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(54) Title: PLANT GENE SPECIFYING ACETYL COENZYME A CARBOXYLASE AND TRANSFORMED PLANTS CONTAINING SAME

(57) Abstract

DNA sequences of an acetyl Coenzyme A carboxylase from plants are inserted into the genome of plants in sense or antisense orientation in order to inhibit expression of the gene product of the endogenous ACCase gene, resulting in reduced conversion of the enzyme's substrate, acetyl Coenzyme A, to fatty acid synthesis, leaving the substrate available for diversion into other biosynthesis pathways. One such diversion may be accomplished by providing the plant genome with genes specifying the synthesis of polyhydroxyalkanoate polymers.

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PLANT GENE SPECIFYING ACETYL COENZYME A CARBOXYLASE AND TRANSFORMED PLANTS CONTAINING SAME

This invention relates to a plant gene specifying the enzyme acetyl Coenzyme A carboxylase (ACCase) and to plant genomes genetically transformed with the said gene. Particularly, but not exclusively, the invention relates to ACCase genes from plants of the Brassica species, especially Brassica napus (oilseed rape) and control of expression of the gene by Brassica plants which are genetically transformed with the gene or its antisense configuration.

Acetyl Coenzyme A carboxylase is one of the genes involved in the synthesis of oil by oil-producing crops such as oilseed rape. Variation of the expression of that gene leads to alteration in the quantity and/or quality of the oil produced.

An object of the invention is to provide a gene specifying ACCase in plants.

According to the present invention there are provided partial cDNAs specifying ACCase, isolated from seed of Brassica napus, having the nucleotide sequences set forth in Figures 6 and 12, and variations thereof permitted by the degeneracy of the genetic code.

The invention further provides the partial cDNA, isolated from wheat germ, having the nucleotide sequence set forth in Figure 4, and variants thereof permitted by the degeneracy of the genetic code.

Also provided by this invention is the full length genomic DNA specifying ACCase from Arabidopsis thaliana

having the nucleotide sequence set forth in Figure 8, and variants thereof permitted by the degeneracy of the genetic code.

The invention further provides the following clones, inserted in Escherichia coli, strain DHα hosts, which have been deposited with the National Collection of Industrial & Marine Bacteria, 23 St. Machar Road, Aberdeen, AB2 1RY, United Kingdom, on 25th March 1993, under the provisions of the Budapest Treaty on the Deposit of Microorganisms for Patent Purposes, details of which are as follows:

1. Plasmid pK111, Accession No. NCIB 40553
2. Plasmid pKLU81, Accession No.NCIB 40554
3. Plasmid pRS1, Accession No. NCIB 40555

The present invention also provides genetically transformed plants, plant cells and plant parts, containing a DNA of the invention or fragment thereof in sense orientation or a complete or partial sense or antisense variant thereof.

It is preferred that the plant be of a species which produces substantial quantities of oil, rather than starch. Such plant species are well known and are simply referred to as "oil-seed" crops and include, oilseed rape, canola, soya and sunflower. Methods for the genetic transformation of many oil crops are known; for example, transformation by Agrobacterium tumefaciens methods are suitable for most. Such methods are well-described in the literature and well-known and extensively practised in the art.

In our International Patent Application Number WO 92/19747, published on 12th November 1992, we describe the biosynthesis of polyhydroxybutyrate from the substrate, acetyl-CoA. This activity involves three enzyme- catalysed steps. The three enzymes involved are

β-ketothiolase, NADP linked acetoacetyl-CoA reductase, and polyhydroxybutyrate synthase, the genes for which have been cloned from Alcaligenes eutrophus (Schubert et al, 1988, J Bacteriol, 170). In our international application we describe the cloning of these three gene into oil-synthesising plants.

However, the synthesis of fatty acids which are the building blocks of plant oils utilise the substrate acetyl Coenzyme A which is the same substrate required by the polyhydroxyalkanoate genes. By virtue of the present invention we provide means for down-regulating the fatty acid synthesis by inhibiting ACCase thereby leaving the acetyl CoA available for conversion to polyhydroxyalkanoates.

Methods for the regulation of gene expression are well-known in the art. Two principal methods are commonly employed, these being referred to loosely as "sense" and "antisense" regulation. In antisense regulation a gene construct is assembled which, when inserted into a plant cell, results in expression of a messenger RNA which is of complementary sequence to the messenger produced by a target gene. The theory is that the complementary RNA sequences form a duplex thereby inhibiting translation to protein. The complementary sequence may be equivalent in length to the whole sequence of the target gene but a fragment is usually sufficient and is more cnvenient to handle. In sense regulation a copy of the target gene is inserted into the plant genome. Again this may be a full length or partial sequence. A range of phenotypes is obtained from which individuals in which the expression of the protein encoded by the target gene is inhibited may be identified and isolated as may individuals where expression of the gene product is increased. Sense regulation using

partial sequences tends to favour inhibition. The mechanism is not well understood. Reference is made to European Patent Application No. 140,308 and United States Patent 5,107,065 which are both concerned with antisense regulation and International Patent Application No. WO 90/12084 which describes sense regulation. The invention permits the following genetic modifications to be effected:

1. The clones of the invention may be used to probe plant DNA (genomic or cDNA libraries) to obtain homologous sequences. These may be truncated or full length cDNAs or genomic DNAs for ACCase genes from, for example, wheat, or oil crops such as rape, canola, soya, sunflower, maize, oil palm and coconut.

2. Partial cDNAs of rape seed ACCase may be used in conjunction with a plant-recognised promoter to create an expression cassette (partial sense or antisense) for use in transforming rape plants to down-regulate production of the ACCase enzyme. This will give plants with a lower oil content or oil of altered quality. The same cassette can be used to down-regulate the production of ACCase enzyme in other plants of the Brassica species. cDNAs isolated from other crops can be used to create expression cassettes (partial, sense or antisense) for use in transformation of these crops in order to modify the oil content.

Down-regulation of oil synthesis (in rape or other oil crops) can be used to divert the substrate, acetyl Coenzyme A, into synthesis of alternative storage materials such as starch, protein, or novel polymers introduced by genetic modification, for example polyhydroxyalkanoates.

3. Full length clones of rape or Arabidopsis ACCase DNA can be used to create expression cassettes, either

with powerful promoters, or by inserting extra gene copies, to promote over-expression of ACCase in rape or other oil crops, leading to plants with enhanced oil content in the seed. The ACCase DNA may also be put under the control of a seed-specific promoter such as the napin promoter, which has a different window of expression from the ACCase promoter during seed development. In this way the period over which ACCase is expressed in the developing seed is extended, and the oil content of the seeds increased.

4. Genomic DNAs of rape ACCase can be used to recover the promoter of the ACCase gene. This promoter can be used to generate RNA in a tissue-specific and developmentally regulated fashion. The promoter so generated may promote the expression of ACCase, or it may control the expression of a gene construct placed after it (for example the structural gene of a different enzyme) which will then be expressed specifically in the developing seed.

5. The full length cDNA and genomic DNA of rape or Arabidopsis ACCase contain a sequence between the translation start site and the N-terminal sequence of the mature protein, known as a "transit peptide" sequence. This directs the gene product to the plastids and is cleaved off during import of the protein into the plastids. This transit peptide sequence may be used in gene fusions to direct different gene products to the plastids.

6. Monocotyledonous plants, such as wheat, barley, maize and rice, are normally sensitive to the aryloxyphenoxy- propionate and alkylketone herbicides to which the dicotyledonous plants are normally resistant. Monocots with resistance to these herbicides may be created by:

(a) transforming ACCase from a dicotyledonous species such as rape and Arabidopsis, into the monocot genome;
(b) overexpression of the ACCase in a monocot; or,
(c) mutagenesis of ACCase and insertion of the mutant gene into a monocot.

7. It is believed that ACCase activity exists in both the plastid and the cytosol. Partial cDNAs of rape seed ACCase of this invention may be used in conjunctipon with a plant-recognised promoter to create an expression cassette (partial sense or antisense) for use in transforming plants to down-regulate production of the cytosolic ACCase. This will alter oil quality by inhibiting production of long chain fatty acids) chain length greater than about C18).

8. A second plastid form of ACCase has been identified in plants. This ACCase is composed of dissociable sub-units for transcarboxylase, biotin carrier protein (BCP) and biotin carboxylase (BC). The transcarboxylase gene is encoded by the chloroplast genome; BCP and BC are nuclear encoded. Sequence homology between the cDNAs of the invention and the BCP and BC may be used to isolate BCP and BC. Sense and antisense constructs may be raised against BCP and BC in order to effect down-regulation of these genes.

9. The cDNAs of the invention may themselves have sufficient homology with the BCP and BC genes to be used directly for the down-regulation of these genes.

We have prepared a poly dT primed cDNA library from developing rape seed and have obtained another from developing wheat embryo. These libraries have been probed with DNA fragments isolated earlier from a partial length maize leaf ACCase DNA (pA3) and partial length cDNA clones specifying rape seed ACCase (pRS1) and wheat germ ACCase (pK111) have thereby been selected and sequenced.

A DNA fragment isolated from the partial length rape ACCase DNA was then used to probe a genomic DNA library prepared from Arabidopsis thaliana and a full length Arabidopsis genomic DNA selected and sequenced.

The sequence of the Arabidopsis genomic DNA was used to generate specific probes by PCR. These were used to screen a random primed cDNA library from rape seed and two further rape ACCase partial cDNAs were thus isolated.

The full length Arabidopsis ACCase genomic DNA may then be used to probe a genomic library from rape and the full length rape ACCase genomic DNA selected and sequenced.

That the clones were indeed of ACCase genes was confirmed as follows:

The deduced amino acid sequence for wheat ACCase cDNA shows complete homology in four regions of sequence to the amino acid sequences obtained from four peptides isolated from the ACCase enzyme purified from wheat embryo. The deduced amino acid sequence shows high homology with both the rat and chicken ACCase genes. High homology at the amino acid level with maize leaf ACCase was found, with two sections of 48 amino acids completely conserved.

The deduced amino acid sequence from the rape seed partial cDNA (pRS1) sequence shows high homology to the sequences of the maize leaf cDNA and the chicken, rat, yeast and algal ACCase genes.

The deduced amino acid sequence from the Arabidopsis genomic DNA shows high homology with the rat, chicken and yeats ACCase genes. High homology with the amino acid sequence of the rape seed ACCase partial cDNA (pRS1) was found, with one section of 48 amino acids almost completely conserved.

The invention will now be described with reference to the accompanying drawings which show:

Figure 1 shows the elution profiles of wheat embryo ACCase from Q-Sepharose (Figure 1A) and Blue-Sepharose (Figure 1B) during purification of the enzyme. The dotted line represents the sodium chloride gradient concentration and the activity of ACCase, represented by the boxes, was measured as described hereinbelow;

Figure 2A shows an SDS PAGE gel of wheat embryo ACCaseshowing the alteration in mobility caused by the binding of streptavidin. Lane 1 contains 500ng myosin (200kDa); lane 2 contains 10μl Post Blue-sepharose material without Streptavidin; and, lane 3 contains 10 μlPost Blue-sepharose material with streptavidin. ACCase is indicated by asterisks (*) at its normal migration and that of the ACCase/streptavidin complex respectively.

Figure 2B shows an SDS PAGE gel of purified wheat embryo ACCase, with the 220kd band taken for sequencing indicated. Lane 1 contains 1μl Post Blue-sepharose material and lane 2 contains 10 μl Post Blue-sepharose material;

Figure 3 shows a comparison of four sections of amino acid sequence deduced from the pK111 wheat ACCase cDNA with the amino acid sequences obtained from four peptides isolated from the purified wheat embryo ACCase enzyme;

Figure 4 shows the sequence of the sense strand of the wheat embryo ACCase clone pK111, with three-phase translation shown. The sequences homologous with the peptide amino acid sequences are underlined;

Figure 5 shows a dot matrix plot of the deduced amino acid sequence of wheat ACCase clone pK111 against that of the maize ACCase clone pA3;

Figure 6a shows the derived amino acid sequence from the rape cDNA encoding the transcarboxylase domain of ACCase. The amino acid sequence is translated from the first open reading frame shown pictorially. The full

vertical lines represent stop codons and the half vertical lines ATG sequences. Figure 6b shows the nucleotide sequence of the cDNA clone pRS1, corresponding to the trans carboxylan domain of ACCase.

Figure 7 shows the rape transcarboxylase domain comparison with known ACCase sequences. The Dot Matrix (DNA Strider, Stringency 9 Window 21) of derived rape ACCase amino acid sequence (transcarboxylase domain) is compared against rat, yeast and algal (Chlorella) ACCase.

Figure 8 shows the 5′ sequence from the sense strand of the Arabidopsis genomic subclone pKLU81, with three phase translation shown.

Figure 9 shows the 3′ sequence from the sense strand of the Arabidopsis genomic subclone pKLU81, with three-phase translation shown.

Figure 10 shows a comparison of the Arabidopsis pKLU81 5′ translated open reading frame with the sequences of rat and chicken ACCase genes obtained from SWISSPROT database.

Figure 11 shows the assignment of domain order to higher plant ACCase. Figure 11A is a schematic diagram showing the yeast ACCase domain orders relative to the sequenced regions (hatched boxes) of the Arabidopsis genomic clone. The areas of sequenced genomic clone are named A-F for easy identification in the text.

In figure 11Bi) the translated open reading frame from area Aii is shown in direct comparison with a region from the biotin carboxylase domain of yeast. Boxed regions represent amino acid identity.

In figure 11Bii) the translated open reading frame corresponding to the biotin binding site in area C is shown in direct comparison with the biotin binding site of yeast. Boxed regions represent amino acid identity.

Figure 11Biii) shows a DNA sequence comparison by dot matrix (DNA Strider, Stringency 15 Window 23) of the rape transcarboxylase domain of ACCase and areas E/F from the

Arabidopsis genomic clone.

Figure 11C shows the nucleotide sequences of Regions A, Aii, B, C, D, E, F, Arabidopsis genomic clone pKLS2.

Figure 12 shows the rape ACCase biotin binding domain sequence.

Figure 12Ai) shows the derived amino acid sequence from the rape cDNA encoding the ACCase biotin binding domain. The actual biotin binding site is shown underlined.

Figure 12Aii) shows the direct comparison of the biotin binding site with that of the corresponding sequence of yeast ACCase. The boxed regions represent amino acid sequence identity.

Figure 12B shows the dot matrix comparison (DNA Strider, Stringency 9 Window 21) of derived rape ACCase amino acid sequence (biotin binding domain) against yeast ACCase.

Figure 12C shows the full combined nucleotide sequence of pRS6 and pRS8.

Figure 13 shows ACCase Southern blot analyses of rape and Arabidopsis genomic DNA. Restrriction endonuclease digested DNA was hybridised to the Arabidopsis ACCase genomic clone by Southern blot. Hybridisation and washing conditions were carried out as described in materials and methods. The blot shown was exposed for 5 days, further exposure provided no extra information. Both λ HindIII and OX 174 HaeIII DNA markers (indicated on the left hand side) were run on the same 1% gel and viewed by ethidium bromide staining/UV.

Figure 14 shows Northern blot analysis of rape ACCase. In Figure 14A the graph shows the oil content as total fatty acid (mg/seed) in relation to the stage of rape embryogenesis. Details of the analysis method are presented in materials and methods. The three Northern blots shown, relating to the different stages of embryogenesis, are all derived from the same blot after

successive stripping. The probes used were as indicated in the text and the amount of polyA+ RNA was 5μg for each stage. Hybridisation and washing conditions were as in materials and methods. Exposure was for 7 days.

In Figure 14B the probe used in the Northern blot shown was the rape transcarboxylase domain cDNA derived from an embryo library. 1μg of poly A+RNA from 29 days post anthesis embryos and young leaf was used for the blot. Hybridisation and washing conditions were as in materials and methods. Exposure was for 7 days. Molecular weight markers were viewed by ethidium bromide/UV.

MATERIALS AND METHODS

1.0 Protein purification and amino acid sequence data.

1.1 Assay for ACCase

Acetyl CoA Carboxylase activity was assayed by incorporation of radioactivity from ^{14}C- bicarbonate into non-volatile malonyl CoA (Hellyer et al 1986).

1.2 SDS Poly-Acrylamide Gel Electrophoresis

All SDS PAGE gels consisted of a 3% stacking gel with a 7.5% running gel on a mini Biorad Protean gel kit unless otherwise stated. The buffer system used was that of Laemmli et al (1970) unless otherwise stated. All gels used in separating peptides for sequencing were pre-run in the presence of 200μM thioglycolic acid in the running buffer.

2.0 Cloning for Wheat/Rape/Arabidopsis ACCase

2.1 Preparation of competent XL1-Blue and KW251

Escherichia coli cells XL1-Blue and KW251 cells were grown overnight in 50 ml LB media/0.2% Maltose/50μg/ml Tetracycline/10 mM $MgSO_4$. The cells were spun down at 3000 g for 10 mins and the cell pellet taken up in 2.5 ml 10 mM $MgSO_4$ and stored at 4°C. Cells were used fresh for primary screening and no older than one week for subsequent screening.

2.2 cDNA Libraries

2.2.1 Wheat

The cDNA library used (gift of Dr Charles Ainsworth, Wye College, London) was generated using the pooled RNA from whole developing grain of Chinese Spring harvested at 3,5,7,10,15,25,30 and 35 days post anthesis. The cDNA was cloned into the EcoRI/XhoI site of λ-ZAP II (Stratagene) and the host bacteria used was XL-1Blue (see 2.1 for preparations of cells).

2.2.2 Rape

(i) cDNA Library from polyA+ RNA

The cDNA library used was generated using the mRNA isolated according to the method of Logemann et al (1987) from mid stage developing Jet neuf rape embryos (harvested at approximately 35 days days post anthesis). The 1st strand synthesis was carried out using poly dT primers according to the manufacturers instructions (Amersham International). The resulting cDNA generated was cloned into the EcoRI/XhoI site of λ-ZAPII as recommended by the manufacturers (Strater gene). The host bacteria used was XL-1Blue (see 2.1 for preparation of cells).

ii) Random primed library

5 μg of poly A+ mRNA from 35 day old (Post anthesis) Jet neuf rape embryo was used for the construction of a random primed cDNA library. The double stranded cDNA was prepared using a 1 in 10 dilution of pd(N)6 primers (0.74μg/ul) according to the instructions provided with Time Saver™ cDNA synthesis kit (Pharmacia). The library was prepared in λZapII and packaged with Gigapack II Gold packaging extract (Stratagene). The host E.coli strain used was XL-1 Blue (Stratagene).

2.3 Genomic Libraries

The Arabidopsis thaliana library used (a gift from Dr John Cowl, John Innes Institute, Norwich) was derived from leaf total DNA in λ FIX II and the host bacteria used was E.coli KW251 (see 2.1 for preparation of cells).

2.4 Probe preparation and labelling

Plasmid DNA from pA3/DH5α (ICI derived) and pRS1/DH5α (see results for a description of pRS1) was prepared by the Quagen tip method. Probe for the screening of Wheat and Rape cDNA libraries was generated by the digestion of 10μg pA3 with 20 U EcoRI or Hind III (New England Biolabs). The fragment isolated from the probe was 2.7 and 1.54 kb in length respectively. Probe for the screening of the Arabidopsis genomic library was generated by a Xho I/Pst I (10 U of each) double digest of 10μg pRS1 to give an isolated fragment size of 1.2 kb. All digests were carried out in Pharmacia's "one-Phor-All Buffer PLUS" at 37°C for 3 hours. Digests were separated by 1% TAE buffered agarose gel electrophoresis and the required fragments cut out from the gel. The DNA was obtained from the gel slice using the method recommended by Geneclean II (Bio 101). DNA concentration was determined by spectrophotometry.

The probes (200-300 ng) were radio-labelled with p^{32}αdCTP using the Megaprime kit as recommended by the manufacturers (Amersham International) to a level of 5 x 10^9 dpm/μg. Un-incorporated label was removed using Biospin chromatography columns (Biorad).

Just before use for hybridisation the radio-labelled probe was boiled for 5 minutes and placed on iced water for 2 minutes before being added to hybridisation buffer at 65°C.

2.5 cDNA library primary screening

For the Wheat cDNA library 300,000 pfu's and the rape random primed and poly dT primed cDNA library 150,000 pfu's were added to 2 ml of competent XL1-Blue cells (150,000 pfu's/2 ml) mixed and incubated at 37°C for 20 minutes. The culture was then added to 30 ml top agarose (150,000 pfu's/ 30 ml) which had been melted and held at

50°C, mixed briefly and poured onto pre-warmed (37°C) large LB plates (243 x 243 x 18 mm). Plates were left at room temperature for 10 minutes and incubated overnight at 37°C. The plates were finally incubated at 4°C for 30 minutes.

Square sheets of nitrocellulose were carefully placed onto the surface of each plate and allowed to soak in for 30 seconds, pealed off and placed onto 3 mm blotting paper soaked in denaturing buffer (1.5 M NaCl, 0.5 M NaOH) for 2 minutes. To neutralise the filters each was subsequently placed for 5 minutes onto 3 mm paper soaked in neutralising buffer (1.5 M NaCl, 0.5 M Tris pH 7.4) and finally for 5 minutes on 3 mm paper soaked in X2 SSC. A second lift of 2 minutes was also carried out and treated in the same way. To immobilise the blotted DNA each filter was placed in a vacuum oven for 30 minutes.

The filters were incubated in pre-hybridisation buffer (50 mls X6 SSC, X1 Dendhart's, 0.5% SDS, 0.05% sodium pyrophosphate, 50$\mu g/m^1$ herring sperm DNA with constant mixing for 3 hours at 65°C at which point the buffer was discarded. The radio-labelled probe (see 2.4) was added to 10 ml hybridisation buffer (50 mls X6 SSC, X1 Dendhart's, 0.5% SDS, 0.05% Sodium Pyrophosphate, 1mM EDTA) previously equilibrated to 65°C. The filters were incubated with constant mixing for 14 hours at 65°C and the hybridisation buffer/probe removed but retained at -20°C for the subsequent screens.

To wash off the un-bound probe the filters were washed 4 times with X1 SSC, 0.1% SDS for 30 minutes at 65°C. Filters were air dried and exposed to film overnight. Positive plaques were located and pulled out from the plate using the wide end of a 1 ml gilson tip. Only plaques that showed up positive on both lifts (30 seconds and 2 minute lifts) were used. The plug was placed into

500 µl SM buffer with 10µl chloroform and incubated at room temperature for 2 hours with occasional mixing. The suspension was spun for 5 minutes on a bench top centrifuge and the supernatant containing the pfu's retained.

2.6 Genomic library primary Screening

The methods used were as already described (see 2.5) but in a genetic manipulation isolation unit 2 x 10^4 were screened in total on 2 plates.

2.7 cDNA and Genomic Secondary Screening

50-200 pfu's in 200µl SM buffer were added to 200µl of competent XL1-Blue cells mixed and incubated at 37°C for 20 minutes. The culture was then added to 3 ml melted top agarose at 50°C, mixed briefly and poured onto pre-warmed 37°C) small LB plates (850 mm diameter). Plates were held at room temperature for 10 minutes and incubated overnight at 37°C. The plates were finally incubated at 4°C for 30 minutes.

Pre-hybridisation and hybridisation was carried out in the same way as that in the primary screen (see 2.5), using the same probe/hybridisation buffer boiled for 5 minutes before use.

The procedure for lifting, preparing, probing, washing and exposing the nitro-cellulose filters was essentially the same as that already described (see 2.5).

The positive plaques were removed as a plug using the wide end of a 200µl Gilson tip, placed into 500µl SM buffer with 10µl chloroform and incubated at room temperature for 2 hours with occasional mixing. The suspension was spun for 5 minutes on a bench top centrifuge and the supernatant containing the pfu's retained.

2.8 cDNA and Genomic tertiary screening

The method was essentially the same as that for the

secondary screen (see 2.7) using only 10-20 pfu's per plant. Exposure of the nitrocellulose filters was only required for 2 hours in this instance.

2.9 Isolation of DNA from positive plaques

Plasmid rescue for cDNA clones was carried out as described by the Stratagene protocol for "in vivo excision of pSK from λ-ZAPII clones". The DNA from the pSK derived clones was prepared in large quantities using the Quagen tip method.

2.10 Preparation of Genomic DNA from positive plaques

One positive plaque was removed from a plate of the positive pfu's from the tertiary screen and incubated with 500 μl fresh KW 251 cells (see 2.1 for method of cell preparation) at 37°C for 20 minutes. Pre-warmed LB media (50 ml at 37°C) was added in addition to 500 μl 1 M $MgSO_4$ and incubated with mild shaking at 37°C for 5-7 hours. Following the 5-7 hours, 250 μl Chloroform was added to the culture and incubated for a further 15 minutes at 37°C. Cell debris was spun out at 10,000 g and DNase/RNase added to the supernatant to a final concentration of 1 μg ml^{-1} and further incubation at 37°C for 30 minutes. 5 g Polyethylene Glycol 8000/3.2 g NaCl was added slowly to the supernatant at 4°C overnight with constant stirring.

The resultant suspension was pelleted at 10,000 g (4°C) and taken up in 5 ml 20 mM Tris-HCl pH 7.4/100 mM NaCl/10 mM $MgSO_4$. The solution was then subjected to 3-5 chloroform extraction's and 3-5 1:1 Phenol:Chloroform extraction. To precipitate the DNA an equal volume of isopropanol (-20°C) was added and left on ice for 30 minutes. The precipitated DNA was pelleted at 10,000 g and washed in 70% Ethanol (-20°C) before being pelleted again. The DNA was resuspended in 300 μl $T_{10}E_1$ buffer.

Subcloning was carried out according to the method used by Sambrook et al (1989).

2.11 Sequencing of DNA clones

Sequencing was carried out by the manufacturer's recommended methods for the machine used (Applied Biosystems Inc 373A DNA sequencer). Both forward and reverse primers (-21 m13 and M13RP1) were used initially for all clones. Oligonucleotides (20 mers) were generated and used to further sequence pRS1 (rape ACCase clone). pK111 (Wheat ACCase clone) was subjected to nested deletions by the recommended method (Pharmacia, "d.s. Nested Deletion Kit") and sequenced by a combination of forward and reverse primers and generated oligonucleotide priming. Computer analysis of DNA sequence was carried out using the SEQNET package from the SERC facility at Daresbury and DNA Strider.

3. Northern blot analysis

Poly A+ mRNA was prepared from either 5g young leaf or 5g embryos harvested at 15, 22,29, 36, 42 and 49 days post anthesis using the recommended procedure (Pharmacia mRNA purification kit). 1-5 ug was loaded on to a 1% formamide/formaldehyde agarose gel for electrophoresis. The Northern blot procedure was as described previously (Elborough et al 1994).

4. Southern blot analysis

Total DNA isolated from rape and Arabidopsis leaves (10ug and 2ug/digestion respectively) was digested with EcoR1, HindIII and BamHI separately for 8 Hrs. The DNA was separated by TAE agarose electrophoresis, blotted and hybridised to radiolabelled probe as described by Sammbrook et al.

RESULTS

1.1 Partial purification of ACCase from Wheat germ

Partial purification of Wheat ACCase was carried out

essentially using the method previously described by Egin-Buhler et al (1980) with several modifications.

All operations were carried out at 4°C unless otherwise stated. All buffers used contained 14 mM β-mercaptoethanol and 0.3 mM EDTA.

6 x 25 g of dry Avalon Wheat germ was ground in a coffee grinder for 15 secs. 200 ml 100 mM Tris-HCl pH 7.5 was added to each and polytroned on full speed for 1 minute. The homogenate was stirred for 15 minutes and spun at 20,000g. The supernatant was stirred with 25g wet weight Dowex 50 previously equilibrated with 100 mM Tris-HCl pH 7.5 for 15 minutes. The suspension was filtered through cheese cloth and 10% Polyethyleneimine at pH 7.5 added to 0.03% w/v dropwise whilst stirring. After 15 minutes the suspension was spun again at 20,000 g. Powdered $(NH_4)_2SO_4$ was added to a final saturation of 60% and stirred for 1 hour. After spinning at 20,000g the pellets were resuspended in 100 ml 100 mM Tris-HCl pH 7.5/100m M NaCl. The supernatant was dialysed for 1 hour against 5 litres 100 mM Tris-HCl pH 7.5/100 mM NaCl and subsequently overnight with fresh buffer (5 litres). Powdered $(NH_4)_2SO_4$ was added to a final saturation of 25% and stirred for 1 hour spun at 20,000 g and the supernatant brought up to 70% saturation. After centrifugation the resulting pellet was resuspended in 50 ml 20 mM Tris-HCl pH 7.5, 20 mM NaCl and dialysed with 3 x 1 hour changes against 5 litres 20 mM Tris-HCl pH 7.5, 20 mM NaCl/20% glycerol. The resultant suspension was diluted to a conductivity of $<4.3 \times 10^{-3}$ cm^{-1} and stirred slowly with 150 ml of pre-equilibrated Q-sepharose (in 20 mM Tris-Hcl pH 7.5 20 mM NaCl/20% glycerol) for 2 hours. The unbound protein was removed using a sintered glass funnel and the matrix washed with 10 volumes of 20 mM Tris-HCl pH 7.5, 20 mM NaCl/20% glycerol. The slurry was

packed into a 10 cm diameter Pharmacia column. Protein was eluted from the column using a gradient of 60-500 mM NaCl/20 mM Tris-HCl pH 7.5/20% glycerol (see figure 1A for elution profile) at 100 ml hr^{-1} collecting approx 9 ml fractions. Every other fraction was assayed for ACCase activity, the most active fractions pooled and brought to 50% $(NH_4)_2SO_4$ saturation. The pellet after centrifugation was taken up in a minimal volume (approx 100 ml) of 20 mM Tris-HCl pH 7.5, 5 mM MgCl, 20% glycerol to give >4.6 x 10^{-3} cm^{-1} conductivity. This was incubated with 100 ml pre-equilibrated Blue-sepharose (in 20 mM Tris-HCl, pH 7.5/5 mM MgCl/ 20% glycerol) with mixing for 2 hours. The matrix was washed with 10 volumes of 20 mM Tris-HCl pH 7.5/5 mM MgCl/20% glycerol using a sintered glass funnel. The washed matrix was packed into a 10 cm diameter Pharmacia column and the protein eluted from the column with a 60-500 mM NaCl/20 mM Tris-HCl pH 7.5/5 mM MgCl/20% glycerol gradient (see Figure 1 B for elution profile) at 100 ml 1 hour taking 9 ml fractions. The pooled active fractions (post-Blue-sepharose material) were stored frozen at -70°C.

1.2 Identification of approx. 220 kDa protein as biotin containing

The dominant 220 kDa a band in the post Blue-sepharose material was identified as ACCase by both its ability to change mobility during SDS PAGE in the presence of streptavidin and its estimated molecular weight (Egin-Buhler et al. (1980). SDS PAGE X5 loading buffer (5μl) was added to 20ul post Blue-sepharose material, boiled at 100°C for 2 mins. and 1μl of a 5mM Steptavidin stock added immediately. The solution was incubated at 650°C for 5 mins. and loaded onto an SDS PAGE gel next to myosin (Mr 200kDa) and untreated post Blue-sepharose material sample for comparison (see Fig 2A). Streptavidin

clearly reduced the mobility of the 220kDa band, indicating that it is biotin containing. The only known biotin enzyme with a MW of 220kDa is ACCase.

1.3 Generation and sequencing of wheat ACCase peptides

A sample of post-Blue sepharose material estimated to contain approx. 400pM (80μg) of ACCase, as determined by comparison with known concentration standards, was loaded onto an SDS PAGE prep gel (see 1.3 for method and Fig 2B for appearance of sample). The running buffer was fresh and had a reduced level of SDS (0.035% SDS). Chromaphor green (Promega) was added at 1:1000 dilution to the upper tank during electrophoresis to allow the visualisation of protein. the ACCase protein band at approx. 220 kDa was cut out of the gel, frozen and stored at -20°C overnight. The gel slices were trimmed of excess acrylamide and loaded on to one well of a 3mm thick large Biorad Protean gel. The gel slices once loaded were overlaid with Endoproteinase LysC (Promega) at 6.5% protein concentration in 50% glycerol/0.125M Tris pH 6.8,/0.1%. SDS/3% B-mercaptoethanol/0.005% Bromophenol Blue. The gel was run until the protein was at the stacker interface at which point electrophoresis was stopped for 1hr at room temperature. Electrophoresis was resumed until the dye front reached the bottom of the gel. Peptides were semi-dry blotted into ProBlot (Applied Biosystems Inc.) according to manufacturers instructions. Rapid Coomassie staining of the blot (according to ProBlot instructions) identified peptide fragments which were excised from the membrane and loaded onto an ABI 477A pulse liquid protein sequencer. Sequence data was obtained at an amino acid level of 10-20pM (see Fig 3).

Sequence data was obtained for 4 peptides, yielding stretches of N-terminal amino acid sequence of 17, 18, 9 and 20 amino acids (Fig 3).

2. ACCase clone isolation and sequencing

2.1 Wheat ACCase cDNA

A wheat cDNA library was probed with a 2.7 kb EcoR1 fragment, and a 1.54 kb HindIII fragment of the maize partial cDNA clone pA3 which contains 4.5 kb of the 3' maize ACCase. This yielded a 1.85 kb clone inserted between the Eco R1 and XhoI site in the multi cloning cassette of pSK. The DNA was recovered by plasmid rescue in the host strain DH5α. This clone was denoted pK111.

The nucleotide sequence data of this partial cDNA, with the derived amino acid sequence from the three reading frames is shown in Figure 4.

Figure 4 also shows that sections of pK111 show complete homology with the amino acid sequence of the 4 peptides isolated from the purified wheat germ enzyme, providing good evidennce that the cDNA does indeed code for wheat embryo ACCase.

A dot matrix comparison of the deduced amino acid sequence from the largest open reading frame against that of the maize ACCase is presented in Figure 5. pK111 showed 82.33% homology with the maize cDNA at the nucloetide level and 88.17% similarity/78.44% identity at the amino acid level.

In addition the deduced amino acid data of the wheat cDNA showed large homologous regions with the known sequences of rat (62%) and yeast (62%) ACCase.

2.2 Isolation of a partial rape ACCase cDNA encoding the transcarboxylase domain.

Although ACCase has been purified from rape embryo, the amounts obtained were not amenable to protein sequencing. To study ACCase at the sequence level we needed to isolate its cDNA. A rape embryo derived poly dT primed λZapII library was screened with the partial wheat ACCase cDNA previously isolated. A hybridising cDNA of 2.5

kb was taken through three rounds of screening and plasmid rescued (pRS1). The clone was fully sequenced in both directions by a combination of nested deletions and dye primer sequencing. The cDNA sequence has been submitted tc EMBL (Accession no. X77382). The predicted amino acid sequence from the largest open reading frame is shown in Figure 6. Dot matrix analysis of the cDNA with previously described ACCase sequences showed it to be a partial clone of ACCase corresponding to the transcarboxylase domain (Figure7) . The predicted amino sequence of the rape clone showed sequence identity/similarity levels of approximately 44/61% with the yeast (Al-Feel et al, 1992), rat (Lopez-Casillas et al, 1988) algae (Roessler and Ohlrogge, 1993) and the wheat ACCase cDNA pK111. Since the mRNA contaions a polyA tail and was obtained from the poly A fraction it is probable that the ACCase cDNA isolated was nuclear encoded.

2.3. Isolation of the Arabidopsis ACCase genomic clone and further rape cDNA cloning

The average insert size of our rape poly dT primed cDNA library, described above, was approximately 2-2.5kb. Therefore it was unlikely that the library would contain much more 5′ cDNA. To obtain more 5′ sequence a random primed library from rape embryo mRNA was constructed. Having made a suitable library there were two strategies available for cloning more 5′ cDNA i) screen using the 5′ region of our cDNA, or; ii) screen using 5′ probes from a genomic clone. We chose the second option. The strategy was to clone the ACCase genomic gene, identify the open reading frames by sequence comparison and generate specific probes by the use of PCR. Since Arabidopsis is related to rape and has a smaller genome we chose to obtain the genomic clone from Arabidopsis. Previous data from this laboratory had shown that Arabidopsis DNA

sequences are highly homologous to those of rape (data not shown). Screening a λ FixII Arabidopsis genomic library with a 1.2 kb Xhol/Pstl fragment of the rape ACCase cDNA pRS1 yielded two independent genomic clones which hybridised strongly to the pRS1 ACCase probe. These were denoted λAYE4 and λAYE8. λAYE8 was subcloned to produce two plasmids : pKLU81, a 5.3 kb subclone in the EcoR1 site of pGEM 3ZF+ ; and pKLS2, which was excised from the λ clone by a partial Sal1 digest and subcloned into pSK+.

The pKLU81 subclone, considered to be a partial length genomic clone, was partially sequenced from the 5' and 3' ends. Therefore two sets of data are presented for the 5' and 3' sequences from the same clone. The nucleotide sequences, with the derived amiono acid sequences from the 3 reading frames are shown in Figures 8 and 9. A data base search (Swissprot) using the derived amino acid sequence from the 5' 0.56kb DNA sequence showed 40% identity with chicken and rat ACCase (Figure 10).

The genomic clone (pKLS2) was extensively subcloned through a combination of EcoRI/SalI/XbaI/HindIII digests, and partially sequenced by both Dye primer and Dye terminator chemistry. We found that intron-exon boundaries could not be allocated without cDNA data. We therefore opted to sequence only enough of the genomic clone to allow generation of open reading frame probes for cDNA screening. The full sequence data obtained is shown schematically in figure 11A (hatched areas A,Aii,B,C,D,E and F) and has been deposited with the EMBL data base (accession no's X77375-X77381).

To map the ACCase activity domain order, within the genomic clone, the open reading frame sequences from the different sequenced areas were compared with the first two domains of the full length yeast cDNA (Figure 11Bi and ii), and the rape transcarboxylase domain (figure 11Biii).

Homology was sufficient to allow us to assign the same order of domains to the Arabidopsis gene as that of yeast ACCase shown in figure 11A ie: [Biotin carboxylase-Biotin binding-Transcarboxylase].

Sequence data from an open reading frame at the 5' end of the genomic clone (area Aii) showed a marked homology (49.5/64% identity/similarity at the derived amino acid level) with the 5' region of yeast ACCase (see Figure 11Bi). The 3' end of the cloned genomic fragment (19kb) was sequenced and shown to be homologous to the 3' end of the rape 2.5kb cDNA clone isolated from the poly d'T primed mRNA library (figure 11Biii). Since we had approximately 1.3kb 5' to area Aii we reasoned that it was likely that pKLS2 was the full length genomic clone. The pKLU 81 subclone was a partial length genomic clone corresponding to a portion of the sequence of pKLS2.

Since the Arabidopsis genomic clone showed a high degree of homology to the rape cDNA isolated (86% identity in the exons of areas E and F) it was clear that the genomic clone could be used to isolate further rape cDNA's. We generated a specific probe via PCR of area C within the genomic clone and used it to screen the random primed library generated from rape embryo mRNA. Two cDNA clones (pRS8 and pRS6 containing 2.0kb and 1.1 kb cDNA respectively) were isolated and sequenced. The cDNA from each was shown to overlap. The full combined derived amino acid sequence (pRS8/6 2.38 kb cDNA size) is presented in figure 12Ai (EMBL accession no X77374). The sequence analysis of the clones showed significant homology with that of yeast (39/58 % identity/similarity), rat (38/59% identity/similarity) and algal (34/54% identity/similarity) ACCase. Within the cDNA sequence is the highly conserved biotin binding site [Val-Met-Lys-Met], shown in figure 12Ai as the underlined region.

Direct comparison with yeast biotin binding site is shown in figure 12Aii. Interestingly the sequence also showed homology at it's 5' end with the 3' portion of the yeast biotin carboxylase domain. This data demonstrated that the domain order in rape [Biotin carboxylase-Biotin binding-Transcarboxylase] is consistent with the domain assignment of Arabidopsis.

3. Southern blot analysis

Since it was not known how many genes for ACCase are present in rape and Arabidopsis, total DNA was analysed by Southern blotting. Both rape and Arabidopsis total DNA was digested with three separate restriction enzymes and blotted. The Arabidopsis genomic clone indicated that ACCase genes would most likely be relatively large. The size dictated that it was not possible, using partial cDNA's as probes, to gain an accurate estimate of the gene copy number by Southern blot. The blot was therefore hybridised to the full Arabidopsisg genomic clone 19kb) labelled by random primed labelling. The sum of the Arabidopsis bands that hybrised to the probe was approximately 20kb (figure 13). Since the genomic clone is approximately 19kb, and showned a similar pattern when digested with the same enzymes (results not shown), we deduced that there is only the one gene present in Arabidopsis. Although the rape profile is more complicated it can be seen that it consists of a relatively small gene family (see figure 13).

4. Northern Blot analysis

The expression of ACCase during rape embryonic development was examined by Northern blotting using the 2.5 kb rape cDNA clone as probe. The blotrs contained 5 ug rape poly A+ m RNA prepared from a set of staged embryos taken from Brassica napus Jet Neuf at 15, 22, 29, 35,42 and 49 days post-anthesis. Embryos taken from the same

seed set were also analysed for oil content to monitor development. The oil content data is presented (expressed as fatty acid/mg seed) graphically in Figure 14A. The Northern blot was hybridised separately to three successive probes and stripped after each in preparation for the next probe. The three probes used were embryo derived cDNAs for enoyl reductase (1.15 kb), βketo reductase (1.185 kb) and ACCase (2.5 kb). All three cDNAs were highly expressed in seed with maximum expression being coincidental at 29 days post-anthesis (Figure 14A). However it appears that the initial onset of mRNA production occurs in the order enoyl reductase, βketo reductase and ACCase. The profile of all three genes expression during embryogenesis was reproducable in individually probed blots with peak expression occuring at 29 days. The sizes of the hybridising bands were 1.65, 1.7 and 7.5 kb respectively as determined by size markers run on the same agarose gel used for the blot. The level of the ACCase mRNA was relatively lower than that of enoyl reductase and βketoreductase. This may be in part due to the successive stripping of the blot and degradation of the large 7.5 kb message during handling.

A Northern blot comparison of ACCase expression in 29 days post-anthesis embryo and young leaf, using the embryo derived 2.5 kb cDNA as a probe is shown in Figure 14B. The 7.5 kb band that hybridises was approximately five times more abundant in seed than in leaf, as might be expected for ACCase. The size of the full length mRNA (7.5 kb) was consistent with the known size of the full length mRNA for both maize and wheat ACCase.

References

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CLAIMS

1. Partial cDNAs specifying acetyl Coenzyme A carboxylase (ACCase), isolated from seed of Brassica napus, having the nucleotide sequence set forth in Figures 6 or 12, and variations thereof permitted by the degeneracy of the genetic code.

2. A partial cDNA specifying ACCase, isolated from wheat germ, having the nucleotide sequence set forth in Figure 4, and variants thereof permitted by the degeneracy of the genetic code.

3. A full length genomic DNA specifying ACCase from Arabidopsis thaliana having the nucleotide sequence set forth in Figure 8, and variants thereof permitted by the degeneracy of the genetic code.

4. The DNA inserts within the following clones, which have been deposited in Escherichia coli, strain DHα hosts, with the National Collection of Industrial & Marine Bacteria, 23 St. Machar Road, Aberdeen, AB2 1RY, United Kingdom, on 25th March 1993, under the provisions of the Budapest Treaty on the Deposit of Microorganisms for Patent Purposes, details of which

are as follows:
Plasmid pK111, Accession No. NCIB 40553;
Plasmid pKLU81, Accession No.NCIB 40554, and,
Plasmid pRS1, Accession No. NCIB 40555

5. A gene construct for use in transforming plants for the purpose of regulating expression of ACCase, comprising a promoter active in plant cells, a structural region encoding mRNA in sense or antisense orientation to one or more domains of the ACCase gene and a 3'untranslated region, whereby transformation of plant cells results in a phenotype characterised by reduced fatty acid production or the production of fatty acids of altered composition.

6. A construct as claimed in claim 5 in which the promoter is a tissue-specific or developmentally regulated promoter.

7. A construct as claimed in claim 6 in which the promoter is the promoter of the napin gene of Brassica napus.

8. A genetically transformed plant, plant cells and plant parts, containing a gene construct as defined in any of claims 5 to 7.

9. A genetically transformed plant and cells and parts thereof, having reduced ability to synthesise fatty acids or having the ability to sythesise fatty acids of altered composition, characterised in that the genome of said plant comprises a gene construct as defined in any of claims 5 to 7.

10. A genetically transformed plant as claimed in claim 9, in which the plant is an oil-synthesising plant.

11. A plant as claimed in claim 10, in which the plant is of the genus Brassicaceae.

12. A plant which is capable of synthesising polyhydroxyalkanoate polymer, characterised in that the the said plant contains within its genome a construct as claimed in any of claims 5 to 7 which reduces the ability of the plant to synthesise fatty acids from acetyl Coenzyme A and in addition genes directing synthesis of polyhydroxyalkanoate from acetyl Coenzyme A.

13. A method for the control of expression of ACCase in a plant comprising stably incorporating into the genome of the said plant by transformation a gene construct as claimed in any of claims 5 to 7.

14. A monocotyledonous plant having increased tolerance of herbicides which inhibit the activity of the ACCase endogenous to monocotyledonous plants comprising a monocotyledonous plant which has stably incorporated within its genome a DNA specifying ACCase, said DNA having been isolated from a dicotyledonous plant possessing a natural tolerance of the said herbicides.

15. A plant as claimed in claim 14 in which the herbicide is selected from the group consisting of arylphenoxy- propionate and alkylketone herbicides.

figure 1

fig 1A ~~Q-sepharose chromatography (Wheat germ)~~

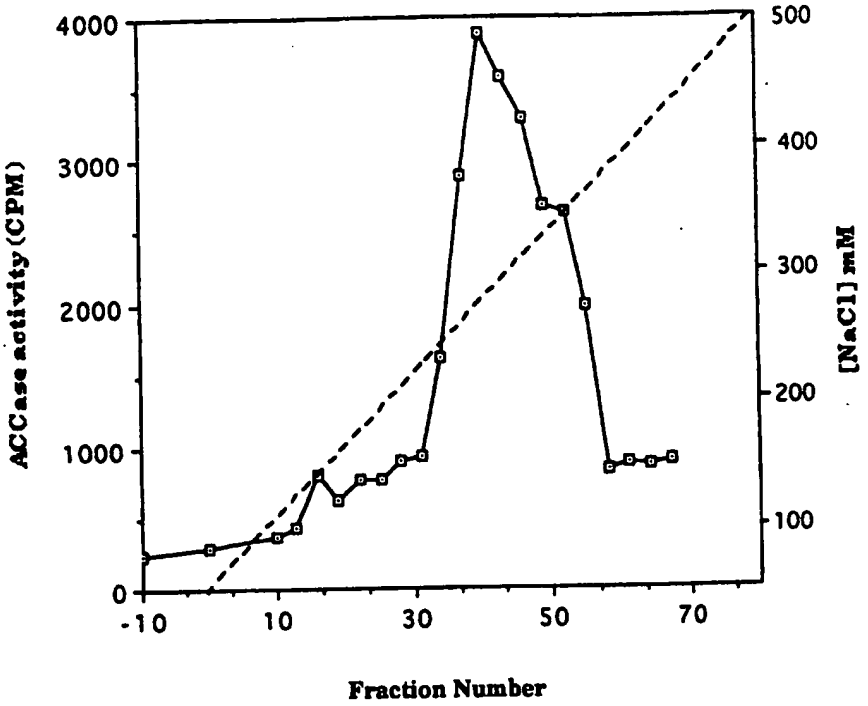
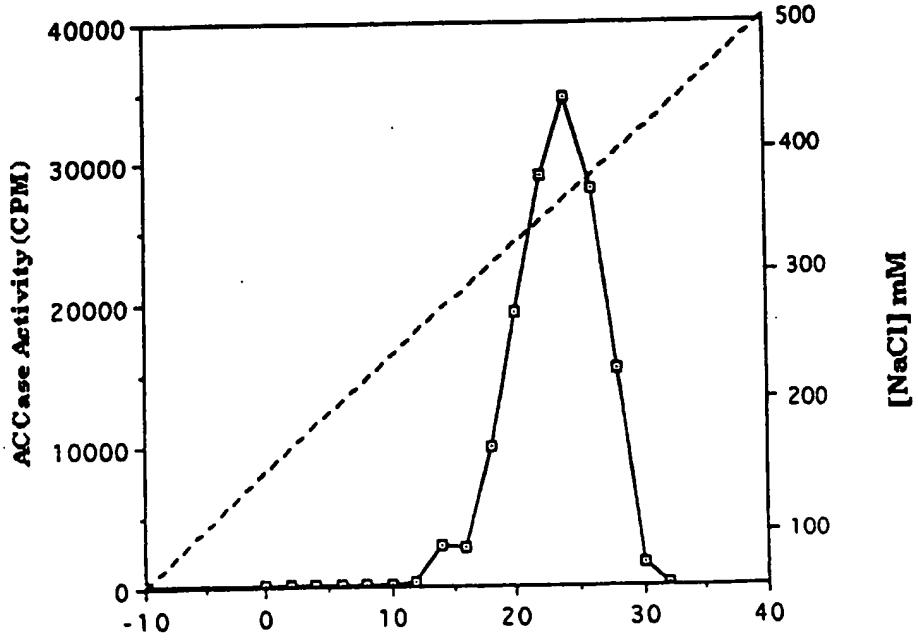


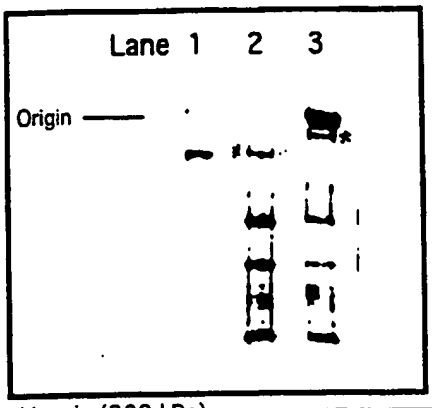
fig 1B ~~Blue-sepharose Chromatography (Wheat germ)~~



~~ACCase elution profiles for both Q-sepharose and Blue-sepharose column~~ chromatography (see 1.2 for methods). The dotted line ~~represents~~ the NaCl gradient concentration and the ~~activity of ACCase~~, represented by the boxes, was measured as in ~~1.1. Only the most active fractions were pooled for the next step in each case.~~

fig 2A

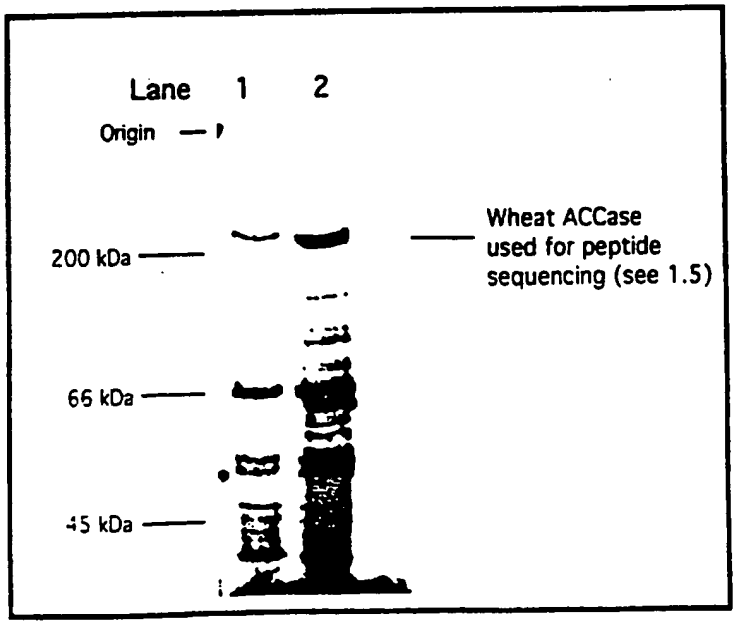
~~Wheat ACCase band shifting on addition of Streptavidin~~



~~Lane 1 contains 500 ng Myosin (200 kDa)~~
Lane 2 contains 10 µl Post Blue-sepharose material without Streptavidin
Lane 3 contains 10 µl Post Blue-sepharose material with Streptavidin (see 1.4)

* ACCase is indicated by the stars at its normal migration and that of the ACCase/streptavidin complex respectively.

fig 2B ~~Wheat ACCase in Post Blue sepharose material~~



~~Lane 1 contains1 µl Post Blue sepharose material (see 1.2 and 1.3 for methods)~~
~~Lane 2 contains 10 µl Post Blue sepharose material~~

FIGURE 3.

fig 3

~~**Authentication of pK111 as Wheat ACCase partial cDNA**~~

~~For methods used see 1.3 and 1.5.~~

AA sequencing from Wheat ACCase MATNGVE?LTVSDDLEG
pK111 deduced AA sequence [81]MATNGVVHLTVSDDLEG

AA sequencing from Wheat ACCase LGGIPVGVIAVETQT?DQ
pK111 deduced AA sequence [161]LGGIPVGXIAVETQTMMQ

AA sequencing from Wheat ACCase NVLEPQGHL
pK111 deduced AA sequence [319]NVXEXQGLI

AA sequencing from Wheat ACCase SIEARKKQLLPLYTQIAIRF
pK111 deduced AA sequence [372]SIEPRKKQLLPLYTQIAVRF

~~In order to authenticate the cDNA clone from wheat (pK111) Amino Acid sequence~~ data was obtained. The direct comparison ~~of AA~~ sequence obtained to that deduced from the ~~cDNA clone~~ is shown. The numbers shown indicate the AA ~~residue site of the sequence within that translated from the cDNA sequence.~~

~~#4. DNA Strider Pl 1.2 ### Wednesday, February 3, 1993 10:14:05 am~~

FIGURE 4

~~WHEAT pKT11 +/- 3-phase Translation~~

DNA sequence 1926 b.p. GAGAACATACAT ... *TCCAGTTTTTT linear

```
1/1                                                                 31/11
GAG AAC ATA CAT GGA AGT GCT GCT ATT GCC AGT GCC TAT TCT AGG GCC TAT GAG GAG ACA
E  N  I  H  G  S  A  A  I  A  S  A  Y  S  R  A  Y  E  E  T
 R  T  Y  M  E  V  L  L  L  P  V  P  I  L  G  P  M  R  R  H
  E  H  T  W  K  C  C  Y  C  Q  C  L  F  *  G  L  *  G  D  I
61/21                                                               91/31
TTT ACG CTT ACA TTT GTG ACT TGA CGG ACT GTT GGA ATA GGA GCA TAT CTT GCT CGA CTT
F  T  L  T  F  V  T  *  R  T  V  G  I  G  A  Y  L  A  R  L
 L  R  L  H  L  *  L  D  G  L  L  E  *  E  H  I  L  L  D  L
  Y  A  Y  I  C  D  L  T  D  C  W  N  R  S  I  S  C  S  T  W
121/41                                                              151/51
GGC ATA CGG TGC ATA CAG CGT ACT GAC CAG CCC ATT ATC CTA ACC GGG TTC TCT GCT TTG
G  I  R  C  I  Q  R  T  D  Q  P  I  I  L  T  G  F  S  A  L
 A  Y  G  A  Y  S  V  L  T  S  P  L  S  *  P  G  S  L  L  *
  H  T  V  H  T  A  Y  *  P  A  H  Y  P  N  R  V  L  C  F  E
181/61                                                              211/71
AAC AAG CTT CTT GGC CGG GAA GTG TAC AGC TCC CAC ATG CAG TTG GGT GGC CCC AAA ATT
N  K  L  L  G  R  E  V  Y  S  S  H  M  Q  L  G  G  P  K  I
 T  S  F  L  A  G  K  C  T  A  P  T  C  S  W  V  A  P  K  L
  Q  A  S  W  P  G  S  V  Q  L  P  H  A  V  G  W  P  Q  N  Y
241/81                                                              271/91
ATG GCG ACA AAC GGT GTT GTC CAT CTG ACA GTT TCA GAT GAC CTT GAA GGT GTG TCT AAT
M  A  T  N  G  V  V  H  L  T  V  S  D  D  L  E  G  V  S  N
 W  R  Q  T  V  L  S  I  *  Q  F  Q  M  T  L  K  V  C  L  I
  G  D  K  R  C  C  P  S  D  S  F  R  *  P  *  R  C  V  *  Y
301/101                                                             331/111
ATA TTG AGG TGG CTC AGC TAT GTT CCT GCC AAC ATT GGT GGA CCT CTT CCT ATT ACA AAA
I  L  R  W  L  S  Y  V  P  A  N  I  G  G  P  L  P  I  T  K
 Y  *  G  G  S  A  M  F  L  P  T  L  V  D  L  F  L  L  Q  N
  I  E  V  A  Q  L  C  S  C  Q  H  W  W  T  S  S  Y  Y  K  I
361/121                                                             391/131
TCT TTG GAC CCA CCT GAC AGA CCC GTT GCA TAT ATC CCT GAG AAT ACA TGT GAT CCT CGT
S  L  D  P  P  D  R  P  V  A  Y  I  P  E  N  T  C  D  P  R
 L  W  T  H  L  T  D  P  L  H  I  S  L  R  I  H  V  I  L  V
  F  G  P  T  *  Q  T  R  C  I  Y  P  *  E  Y  M  *  S  S  C
421/141                                                             451/151
GCA GCC ATC AGT GGC ATT GAT GAT AGC CAA GGG AAA TGG TTG GGG GGC ATG TTC GAC AAA
A  A  I  S  G  I  D  D  S  Q  G  K  W  L  G  G  M  F  D  K
 Q  P  S  V  A  L  M  I  A  K  G  N  G  W  G  A  C  S  T  K
  S  H  Q  W  H  *  *  *  P  R  E  M  V  G  G  H  V  R  Q  R
481/161                                                             511/171
GAC AGT TTT GTG GAG ACA TTT GAA GGA TGG GCG AAG TCA GTA GTT ACT GGC AGA GCG AAA
D  S  F  V  E  T  F  E  G  W  A  K  S  V  V  T  G  R  A  K
 T  V  L  W  R  H  L  K  D  G  R  S  Q  *  L  L  A  E  R  N
  Q  F  C  G  D  I  *  R  M  G  E  V  S  S  Y  W  Q  S  E  T
541/181                                                             571/191
CTC GGA GGG ATT CCG GTG GGT GT* ATA GCT GTG GAG ACA CAG ACT ATG ATG CAG CTC ATC
L  G  G  I  P  V  G  X  I  A  V  E  T  Q  T  M  M  Q  L  I
 S  E  G  F  R  W  V  X  *  L  W  R  H  R  L  *  C  S  S  S
  R  R  D  S  G  G  C  X  S  C  G  D  T  D  Y  D  A  A  H  P
601/201                                                             631/211
CCT GCT GAT CCA GGG CAG CTT GAT TCC CAT GAG CGG TCT GTT CCT CGT *CT GGG CAA GT*
P  A  D  P  G  Q  L  D  S  H  E  R  S  V  P  R  X  G  Q  X
 L  L  I  Q  G  S  L  I  P  M  S  G  L  F  L  X  L  G  K  X
  C  *  S  R  A  A  *  F  P  *  A  V  C  S  S  X  W  A  S  X
661/221                                                             691/231
TGG TTT CCA *AT T*A *CT ACT AAG ACA GCT CAA GCA ATG CTG GAC TTC AAC CGT *AA GGA
W  F  P  X  X  X  T  K  T  A  Q  A  M  L  D  F  N  R  X  G
 G  F  X  I  X  L  L  R  Q  L  K  Q  C  W  T  S  T  X  K  D
  V  S  X  X  X  Y  *  D  S  S  S  N  A  G  L  Q  P  X  R  I
721/241                                                             751/251
TTA CCT CT* TTC ATC CTT GC* AAC TGG AGA GGC TTC T*T GGT GGG CAA AGA GAT CTT TTT
L  P  X  F  I  L  X  N  W  R  G  F  X  G  G  Q  R  D  L  F
 Y  L  X  S  S  L  X  T  G  E  A  S  X  V  G  K  E  I  F  L
  T  S  X  H  P  C  X  L  E  R  L  X  W  W  A  K  R  S  F  *
781/261                                                             811/271
AAA GGA ATC CTT CAG GCT GGG TCA ACA ATT GTT GAG AAC CTT AGG ACA TAC AAT CAG CCT
K  G  I  L  Q  A  G  S  T  I  V  E  N  L  R  T  Y  N  Q  P
 K  E  S  F  R  L  G  Q  Q  L  L  R  T  L  G  H  T  I  S  L
  R  N  P  S  G  W  V  N  N  C  *  E  P  *  D  I  Q  S  A  C
```

FIG 4 (contd)


```
841/281                                                                871/291
GCC TTT GTA TAT ATC CCC AAG GCT GCA GAG CTA CGT GGA GGG GCT TGG GTC GTG ATT GAT
A   F   V   Y   I   P   K   A   A   E   L   R   G   G   A   W   V   V   I   D
 P   L   Y   I   S   P   R   L   Q   S   Y   V   E   G   L   G   S   *   L   I
  L   C   I   Y   P   Q   G   C   R   A   T   W   R   G   L   G   R   D   *   *
901/301                                                                931/311
AGC AAG ATA AAT CCA GAT CGA TTT GAG TTC TaT GCT GAG AGG ACT GCA AAG GGT AAT GTT
S   K   I   N   P   D   R   F   E   F   Y   A   E   R   T   A   K   G   N   V
 A   R   *   I   Q   I   D   L   S   S   M   L   R   G   L   Q   R   V   M   F
  Q   D   K   S   R   S   I   *   V   L   C   *   E   D   C   K   G   *   C   S
961/321                                                                991/331
CT* GAA CC* CAA GGG TTG ATT GA* ATC AA* TTC AGG TCA GAG GAA CTC CAA GAG TGC ATG
X   E   X   Q   G   L   I   X   I   X   F   R   S   E   E   L   Q   E   C   M
 X   N   X   K   G   *   L   X   S   X   S   G   Q   R   N   S   K   S   A   W
  X   T   X   R   V   D   *   X   Q   X   Q   V   R   G   T   P   R   V   E   G
1021/341                                                               1051/351
GGC AGG GTT GAC CCA GAA TTG ATA AAT CTG AAG GCA AAA CTC CTG GGA GCA AAG CAT GAC
G   R   V   D   P   E   L   I   N   L   K   A   K   L   L   G   A   K   H   D
 A   G   L   T   Q   N   *   *   I   *   R   Q   N   S   W   E   Q   S   M   T
  Q   G   *   P   R   I   D   K   S   E   G   K   T   P   G   S   K   A   *   Q
1081/361                                                               1111/371
AAT GGA AGT CTA TCT GAG TCA GAA TCC CTT CAG AAG AGC ATA GAA CCC CGG AAG AAA CAG
N   G   S   L   S   E   S   E   S   L   Q   K   S   I   E   P   R   K   K   Q
 M   E   V   Y   L   S   Q   N   P   F   R   R   A   *   N   P   G   R   N   S
  W   K   S   I   *   V   R   I   P   S   E   E   H   R   T   P   E   E   T   V
1141/381                                                               1171/391
TTG TTG CCT TTG TAT ACT CAA ATT GCG GTG CGG TTT GCT GAA TTG CAT GAC ACT TCC CTT
L   L   P   L   Y   T   Q   I   A   V   R   F   A   E   L   H   D   T   S   L
 C   C   L   C   I   L   K   L   R   C   G   L   L   N   C   M   T   L   P   L
  V   A   F   V   Y   S   N   C   G   A   V   C   *   I   A   *   H   F   P   *
1201/401                                                               1231/411
AGA ATG GCT *CT AAG GGT GTG ATT AAG AAG GTT GTA GAC TGG AAA GAT TCT AGG TCT TTC
R   M   A   X   K   G   V   I   K   K   V   V   D   W   K   D   S   R   S   F
 E   W   X   L   R   V   *   L   R   R   L   *   T   G   K   I   L   G   L   S
  N   G   X   *   G   C   D   *   E   G   C   R   L   E   R   F   *   V   F   L
1261/421                                                               1291/431
TTC TAC AAG AGA TTA CGG AGG AGG ATA TCC GAG GAC GTT CTT *CA AAG GAA ATT AGA GGT
F   Y   K   R   L   R   R   R   I   S   E   D   V   L   X   K   E   I   R   G
 S   T   R   D   Y   G   G   G   Y   P   R   T   F   X   Q   R   K   L   E   V
  L   Q   E   I   T   E   E   D   I   R   G   R   S   X   K   G   N   *   R   C
1321/441                                                               1351/451
GTA AGT GGC AAG CAG TTC TCT CAC CAA TCA GCA ATC GAG CTG ATC CAG AAA TGG TAC TTG
V   S   G   K   Q   F   S   H   Q   S   A   I   E   L   I   Q   K   W   Y   L
 *   V   A   S   S   S   L   T   N   Q   Q   S   S   *   S   R   N   G   T   W
  K   W   Q   A   V   L   S   P   I   S   N   R   A   D   P   E   M   V   L   G
1381/461                                                               1411/471
GCT TCT AAG GGA GCT GAA GCA GCA AGC ACT GAA TGG GAT GAT GAC GAT GCT TTT GTT GCC
A   S   K   G   A   E   A   A   S   T   E   W   D   D   D   D   A   F   V   A
 L   L   R   E   L   K   Q   Q   A   L   N   G   M   M   T   M   L   L   L   P
  F   *   G   S   *   S   S   K   H   *   M   G   *   *   R   C   F   C   C   L
1441/481                                                               1471/491
TGG AGG GAA AAC CCT GAA AAC TAC CAG GAG TAT ATC AAA GAA CTT AGG GCT CAA AGG GTA
W   R   E   N   P   E   N   Y   Q   E   Y   I   K   E   L   R   A   Q   R   V
 G   G   K   T   L   K   T   T   R   S   I   S   K   N   L   G   L   K   G   Y
  E   G   K   P   *   K   L   P   G   V   Y   Q   R   T   *   G   S   K   G   I
1501/501                                                               1531/511
TCT CAG TTG CTC TCA GAT GTT GCA GAC TCC AGT CCA GAT CTA GAA GCC TTG CCA CAG GGT
S   Q   L   L   S   D   V   A   D   S   S   P   D   L   E   A   L   P   Q   G
 L   S   C   S   Q   M   L   Q   T   P   V   Q   I   *   K   P   C   H   R   V
  S   V   A   L   R   C   C   R   L   Q   S   R   S   R   S   L   A   T   G   S
1561/521                                                               1591/531
CTT TCT ATG CTA CTA GAG AAG ATG GAT CCC TCA AGG AGA GCA CAG TTT GTT GAG GAA GTC
L   S   M   L   L   E   K   M   D   P   S   R   R   A   Q   F   V   E   E   V
 F   L   C   Y   *   R   R   W   I   P   Q   G   E   H   S   L   L   R   K   S
  F   Y   A   T   R   E   D   G   S   L   K   E   S   T   V   C   *   G   S   Q
1621/541                                                               1651/551
AAG AAA GTC CTT AAA TGA TCA GAT GAT ACC AAC GCA TCC AAT TCA GAA TGT GCA TGA TAT
K   K   V   L   K   *   S   D   D   T   N   A   S   N   S   E   C   A   *   Y
 R   K   S   L   N   D   Q   M   I   P   T   H   P   I   Q   N   V   H   D   I
  E   S   P   *   M   I   R   *   Y   Q   R   I   Q   P   R   M   C   M   I   S
1681/561                                                               1711/571
CGG TTT CTC TTG AAG TAC ATA TAT AGA *GG ATA CTA TTC GGC TGT AAC CGA CCA TAG CTG
R   F   L   L   K   Y   I   Y   R   X   I   L   F   G   C   N   R   P   *   L
 G   F   S   *   S   T   Y   I   X   G   Y   Y   S   A   V   T   D   H   S   *
  V   S   L   E   V   H   I   *   X   D   T   I   R   L   *   P   T   I   A   D
```

6/25

Fig 4 (contd)

~~Wheat pK111 - 3 phase Translation~~ ~~2/2/03 - 10:14:05 am - Page 3~~

```
1741/581                                                    1771/591
ATC TGA GTC AAC CAT TAT TTT GTA AAA CTT TTT TGC GGT CTT CTC TGT TAT TCG AGG CAA
I   *   V   N   H   Y   F   V   K   L   F   C   G   L   L   C   Y   S   R   Q
 S   E   S   T   I   I   L   *   N   F   F   A   V   F   S   V   I   R   G   K
  L   S   Q   P   L   F   C   K   T   F   L   R   S   S   L   L   F   E   A   K
1801/601                                                    1831/611
AAC TTG TTT TCG GAC GGC TCC GAA TGG TTG ATG AGT GTA GTT GGA AAA AAA GCG GCC GGA
N   L   F   S   D   G   S   E   W   L   M   S   V   V   G   K   K   A   A   G
 T   C   F   R   T   A   P   N   G   *   *   V   *   L   E   K   K   R   P   E
  L   V   F   G   R   L   R   M   V   D   E   C   S   W   K   K   S   G   R   N
1861/621                                                    1891/631
ATT *CT GCA GCC CGG GGG ATC C*C TAG TTC TAG AGC GGC CGC ACC GGG TTG GAG *TC CAG
I   X   A   A   R   G   I   X   *   F   *   S   G   R   T   G   L   E   X   Q
 X   L   Q   P   G   G   S   X   S   S   R   A   A   A   P   G   W   X   S   S
  X   C   S   P   G   D   X   L   V   L   E   R   P   H   R   V   G   X   P   V
1921/641
TTT TTT
F   F
 F
  F
```

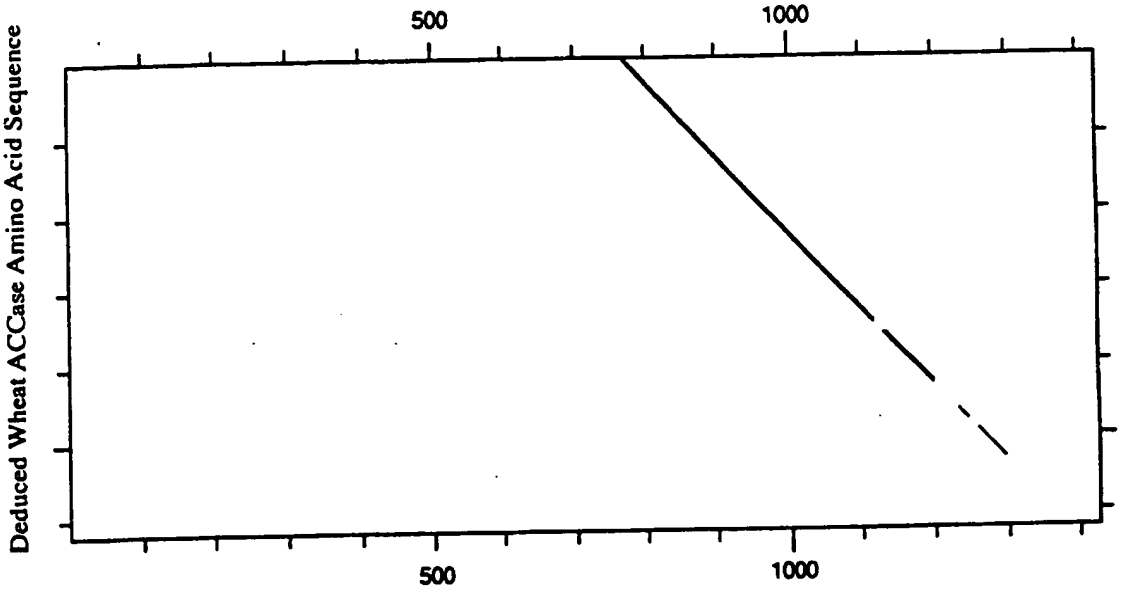
~~The sense strand of the Wheat cDNA sequence for pK111 with 3 Phase translation~~ shown. The 1st phase is the translation from ~~the largest open~~ reading frame. The underlined sequences ~~are those~~ homologous to the Amino Acid data obtained for ~~Wheat ACC ase (see fig 3).~~

FIGURE 5

fig 5

~~Dot Matrix Maize V Wheat Amino Acid sequence~~

~~Deduced Maize ACCase Amino Acid sequence~~



~~Using DNA strider the two sequences were compared by dot matrix (stringency 15, window 23). The wheat sequence used was the 1st phase translation in respect to fig 4. The Maize ACCase sequence was. ~~~~ derived.~~

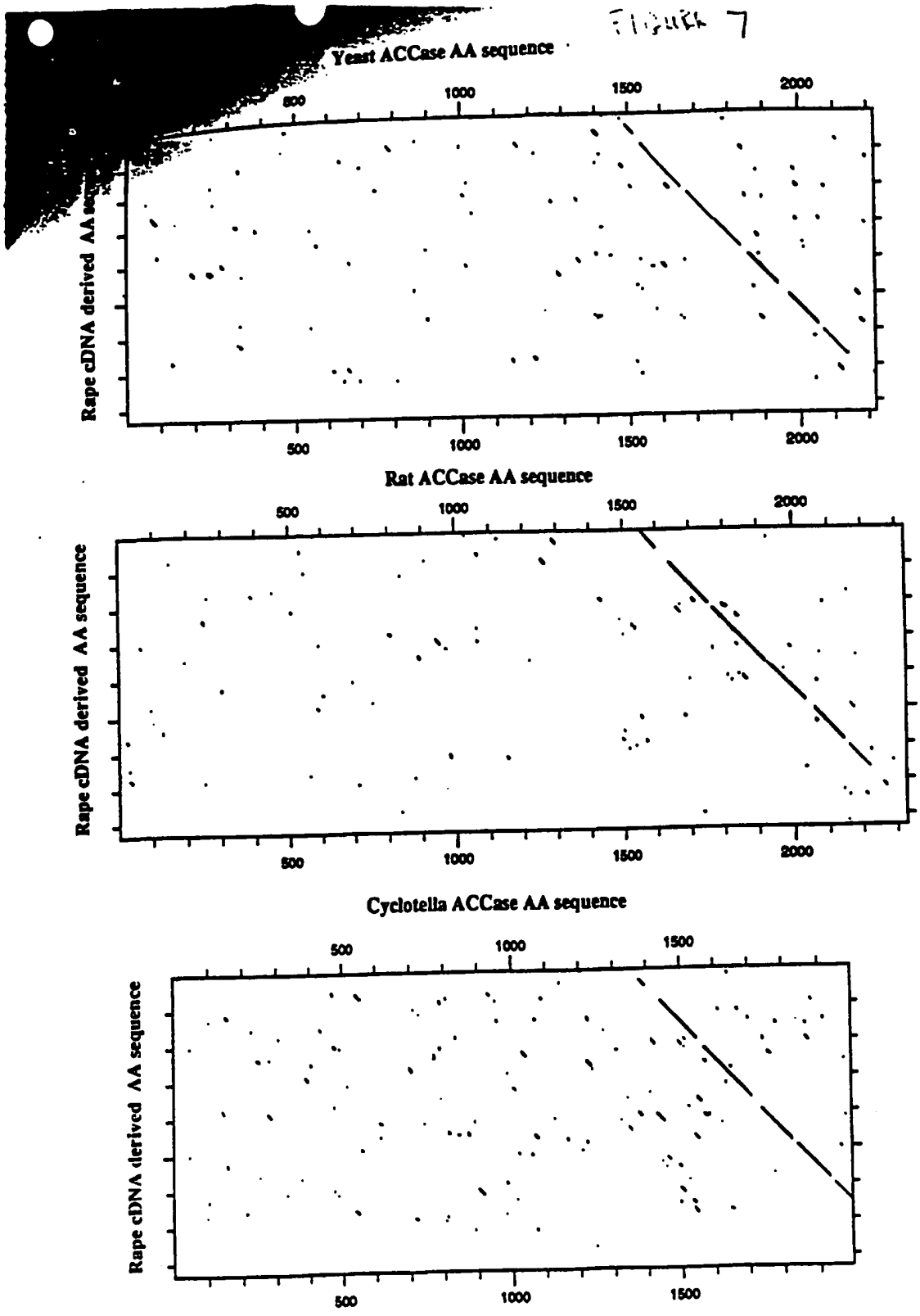
FIGURE 6A

```
          |   10        |   20        |   30        |   40        |   50        |   60        |   70        |   80
    1 ARGRNSLIYH SITKKGPLHG TQINDQYKPL GYLDRQRLAA RRSNITTYCYD FPLAFETALE QFGHYNNREL RNHARVLLSV
   81 LKSLYSPISE GTSLMPVERS PGLNEFGMVA WSLEMSTPEF PMGRKLLIVA NDVTFKAGSF GPREDAFFLA VTELACPKKL
  161 PLIYLAPNSG ARLGVAEEIK ACFKVGWSDE VSPENGFQYI YLSPEDHARI GSSVIAHEIK LPSGETRWVI DTIVGKEDGI
  241 GVENLTGSGP IAGAYSRAYN ETFTLTFVSG RTVGIGAYLA PLGMRCIQRL DQPIILTGFS TLNKLLGREV YSSHMQLGGP
  321 KIMGTNGVVH LTVSDDLEGV SAILDWLSYI PAYVGGPLPV LAPLDPPDRT VEYVPENSCD PRAAIAGVND NTGKWLGGIF
  401 DKNSFIETLE GWARTVVTGR AKLGGIPVGV VAVETQTVMQ IIPADPGQLD SHERVVPQAG QVWFPDSAGK TAQALMDFTR
  481 KSFHCLSLRT GEGFQVGREI FSKEYFRQVA TIVENLRTYR QPVFVYIPKM GELRGGAWVV VDSQINSDYV EMYADETARG
  561 NVLEPEGTIE IKFRTKEMLE CMGRLDPKLI DLKARLQDPN QSEAYTNIEL LQQQIKAREK LLLPVYIQIA TKFAELHDTS
  641 MRMTAKGVIK MCVEWIGSRS FFYKKLNRRI AENSLVKNVR EASGDDLSYK SAMGLIQDWF SKSDIPKGKE EAWTDDQVFF
  721 TWKDNVSNYE LNLSELRPQK LLNPTCRDWK FRRIYRRCHK DLPIF  765
```

Figure 6b

GCACGAGGGAGAAACAGTTTGATTTACCACTCAATTACCAAGAAGGGACCTTTGCATGGAA
CCCAAATCAATGATCAATATAAGCCACTGGGATATCTTGACAGGCAACGTCTAGCCGCAAG
GAGGAGTAACACTACATATTGCTATGACTTCCCGTTGGCATTTGAGACAGCCTTGGAGCAGT
TTGGGCATTACAACAACCGGGAGTTAAGAAACCATGCAAGGGTACTCTTATCAGTGCTAAA
GAGCTTGTATTCTCCAATTTCAGAAGGTACATCTCTTATGCCAGTTGAAAGATCACCGGGTC
TCAATGAGTTTGGAATGGTGGCCTGGAGCCTAGAGATGTCGACTCCTGAGTTTCCTATGGG
ACGGAAGCTTCTCATAGTCGCCAATGATGTCACCTTCAAAGCTGGTTCTTTTGGTCCTAGA
GAGGACGCGTTTTTCCTTGCCGTGACTGAACTCGCATGTCCCAAGAAGCTTCCCTTGATTTA
CTTGGCACCAAATTCTGGTGCCAGACTCGGAGTAGCTGAAGAAATCAAAGCCTGCTTTAAA
GTTGGATGGTCGGATGAAGTTTCCCCCGAAAATGGTTTTCAGTATATATACCTAAGCCCTGA
AGACCATGCAAGGATTGGATCATCTGTCATTGCGCACGAAATAAAGCTCCCTAGTGGGGAA
ACAAGGTGGGTGATTGATACAATCGTTGGTAAAGAAGATGGTATTGGTGTAGAGAATCTAA
CCGGAAGTGGGCCAATAGCGGGCGCTTACTCGAGGGCATACAACGAAACATTTACTTTGAC
CTTTGTTAGTGGAAGAACGGTAGGAATTGGTGCTTACCTTGCCCCCCTTGGTATGCGGTGTA
TACAGAGACTTGACCAGCCGATCATATTGACTGGCTTTTCTACGCTCAACAAGTTACTTGGG
CGTGAGGTCTATAGCTCTCACATGCAACTTGGTGGCCCGAAAATCATGGGCACAAATGGTGT
TGTTCATCTTACAGTCTCAGATGATCTCGAAGGTGTATCAGCGATTCTCGACTGGCTGAGCT
ACATTCCTGCTTACGTTGGTGGTCCTCTTCCTGTTCTTGCCCCGTTAGACCCACCGGACAGAA
CCGTGGAGTACGTTCCAGAGAACTCTTGCGACCCGCGAGCTGCTATAGCTGGGGTTAACGA
CAATACCGGTAAATGGCTTGGCGGTATCTTTGATAAAAATAGCTTTATTGAGACTCTTGAAG
GCTGGGCAAGAACGGTAGTGACTGGTAGAGCTAAACTAGGGGGAATACCTGTAGGAGTTG
TTGCGGTTGAGACACAGACAGTAATGCAGATCATCCCAGCAGATCCAGGACAGCTCGACTC
TCATGAAAGAGTGGTTCCACAGGCAGGGCAAGTCTGGTTTCCTGATTCTGCGGGCAAGACA
GCTCAAGCGCTCATGGATTTCACAAGGAAGAGCTTCCATTGTTTATCCTTGCGAACTGGAG
AGGGTTTTCAGGTGGGCAGAGAGATCTTTTCGAAGGAATACTTCAGGCAGGTTGCGACTAT
TGTAGAAAATCTGAGAACGTATCGGCAGCCAGTGTTTGTGTACATCCCTAAGATGGGAGAG
TTGCGAGGTGGAGCGTGGGTTGTTGTTGATAGCCAAATAAATTCAGATTATGTTGAAATGT
ATGCTGATGAAACTGCTAGGGGGAATGTGCTTGAGCCAGAAGGAACGATAGAGATAAAAT
TTAGAACGAAAGAGATGTTAGAGTGCATGGGAAGGTTAGACCCGAAGCTAATCGATCTCAA
AGCAAGACTGCAAGATCCCAACCAAAGTGAGGCTTATACAAATATCGAGCTCCTCCAGCAA
CAGATTAAAGCCCGAGAGAAGCTTCTCTTACCAGTTTATATCCAAATCGCCACCAAATTTGC
GGAACTTCACGATACTTCCATGAGAATGACTGCCAAAGGAGTGATCAAAATGTGTGTGGAG
TGGATCGGCTCGAGGTCCTTCTTCTATAAGAAGCTCAACCGGAGAATTGCTGAGAACTCTC
TTGTGAAAAACGTAAGAGAAGCTTCAGGAGACGACTTATCGTATAAATCTGCAATGGGTTT
AATTCAGGATTGGTTCTCCAAATCTGACATTCCAAAGGGGAAAGAAGAAGCTTGGACAGAC
GACCAAGTGTTCTTTACATGGAAGGACAACGTTAGTAACTACGAGTTGAATCTGAGCGAAT
TGAGACCGCAGAAACTGTTGAACCCAACTTGCAGAGATTGGAAATTCCGTCGGATCTATCG
GCGCTGCCACAAGGACTTGCCAATCTTCTAAACAAGGTGGAGCCTTCAAGAAGAGAAGAGC
TTGTTGAAGCGCTACGAAAAGTGTTAGGTTGATGTACAAGAGGTCAAGCTTGTGACCCGAG
AAAGATGGTCCTTTGGTGTTGCTTGTGTCCTACGGTGAAAGAAGCTAGTTGGAAATTAGAT
GTGGTCTTTCTTTCTTAAATGTGTTGGCCCGAGCTGTAAATGTTGTTGTAGCGTATAAGTGA
GAATTGCGTAATAATTTATTCAAC

FIGURE 7


Yeast ACCase AA sequence
Rape cDNA derived AA sequence
500
1000
1500
2000
Rat ACCase AA sequence
Rape cDNA derived AA sequence
500
1000
1500
2000
Cyclotella ACCase AA sequence
Rape cDNA derived AA sequence
500
1000
1500

~~##. DNA Strider™ 1.2 ### Thursday, February 4, 1993 3:50:42 pm~~

FIGURE 8

~~pKLUx81 1.1 -> 3-phase Translation~~

~~DNA sequence 565 b.p. CTCTCTGGCAAA ... [illegible] linear~~

```
1/1                                         31/11
CTC TCT GGC AAA TCC CTG GTA TAA TCT ACG TCC TTA TTT CTT ACA GGC AGC GGT TCC TCT
L   S   G   K   S   L   V   *   S   T   S   L   F   L   T   G   S   G   S   S
 S   L   A   N   P   W   Y   N   L   R   P   Y   F   L   Q   A   A   V   P   L
  L   W   Q   I   P   G   I   I   Y   V   L   I   S   Y   R   Q   R   P   L   F
61/21                                       91/31
TCT TTA TCC ATG CAC ACG AAT AAT GTA CTG TCT GTT TCT CTT TAA TTT CGT AGA GAT AAG
S   L   S   M   H   T   N   N   V   L   S   V   S   L   *   F   R   R   D   K
 L   Y   P   C   T   R   I   M   Y   C   L   F   L   F   N   F   V   E   I   R
  F   I   H   A   H   E   *   C   T   V   C   F   S   L   I   S   *   R   *   D
121/41                                      151/51
ACG GTT CTA TGG AAT AGA ACA TGG TGG AGG TTA TGA TTC TTG GCG AAA AAC ATC TGT TGT
T   V   L   W   N   R   T   W   W   R   L   *   F   L   A   K   N   I   C   C
 R   F   Y   G   I   E   H   G   G   G   Y   D   S   W   R   K   T   S   V   V
  G   S   M   E   *   N   M   V   E   V   M   I   L   G   E   K   H   L   L   *
181/61                                      211/71
AGC CTT CCC TTT TGA TTT TGA TAA AGC TCA ATC TAT AAG GCC AAA AGG TCA TTG TGT GGC
S   L   P   F   *   F   *   *   S   S   I   Y   K   A   K   R   S   L   C   G
 A   F   P   F   D   F   D   K   A   Q   S   I   R   P   K   G   H   C   V   A
  P   S   L   L   I   L   I   K   L   N   L   *   G   Q   K   V   I   V   W   L
241/81                                      271/91
TGT ACG TGT GAC AAG TGA GGt aTC CTG ATG ACG GGT TCA AAC CAA CCA GCG GTA GAG TTC
C   T   C   D   K   *   G   I   L   M   T   G   S   N   Q   P   A   V   E   F
 V   R   V   T   S   E   V   S   *   *   R   V   Q   T   N   Q   R   *   S   S
  Y   V   *   Q   V   R   Y   P   D   D   G   F   K   P   T   S   G   R   V   Q
301/101                                     331/111
AGG TAA TGT GAT ATC TGT GGA ATG CAA AGT GAA AGT TCA TTC ACT GAG GAA CTC TGT GGG
R   *   C   D   I   C   G   M   Q   S   E   S   S   F   T   E   E   L   C   G
 G   N   V   I   S   V   E   C   K   V   K   V   H   S   L   R   N   S   V   G
  V   M   *   Y   L   W   N   A   K   *   K   F   I   H   *   G   T   L   W   G
361/121                                     391/131
GTA ACA CTT GTA TGA ACT TGC AAC AGG aGT TGA GTT TTA AGA GCA AGC CAA ATG TGT GGG
V   T   L   V   *   T   C   N   R   S   *   V   L   R   A   S   Q   M   C   G
 *   H   L   Y   E   L   A   T   G   V   E   F   *   E   Q   A   K   C   V   G
  N   T   C   M   N   L   Q   Q   E   L   S   F   K   S   K   P   N   V   W   A
421/141                                     451/151
CGT ACT TCT CTG TCA AGG TAA TTT ATA TCT ATA GaG aCt ctg cta tat aag tgt ttc aca
R   T   S   L   S   R   *   F   I   S   I   E   T   L   L   Y   K   C   F   T
 V   L   L   C   Q   G   N   L   Y   L   *   R   L   C   Y   I   S   V   S   Q
  Y   F   S   V   K   V   I   Y   I   Y   R   D   S   A   I   *   V   F   H   N
481/161                                     511/171
atg *tt taa ttt t*c ggc tac ttt ttt aca gct gtg ggg cac ccg *gt ctt ggt tcc att
M   X   *   F   X   G   Y   F   F   T   A   V   G   H   P   X   L   G   S   I
 X   F   N   F   X   A   T   F   L   Q   L   W   G   T   X   V   L   V   P   F
  X   L   I   X   R   L   L   F   Y   S   C   G   A   P   X   S   W   F   H   L
541/181
tgg aag t*g atg aaa *aa tgt ttt a
W   K   X   M   K   X   C   F
 G   S   X   *   X   N   V   L
  E   X   D   E   X   M   F
```

~~The sense strand of the Arabidopsis sequence for pKLU81 with 3 Phase translation shown. The sequence shown is at the 5' end of the subclone. The 1st phase is the translation from the largest open reading frame.~~

~~#A. DNA Strider™ 1.2 ### Thursday, February 4, 1993 4:02:19 pm~~

FIGURE 9

~~pKLU81 15 -> 3 phase Translation~~

~~DNA sequence 255 b.p. tgcccctggat TGCTTTGTTACT linear~~

```
1/1                                                    31/11
tgc ccc ctg gat ggc atg tgg tgc ttg gag ggt tgt ggt tgc aaa cgt gac agg CCG TAC
C   P   L   D   G   M   W   C   L   E   G   C   G   C   K   R   D   R   P   Y
 A   P   W   M   A   C   G   A   W   R   V   V   V   A   N   V   T   G   R   T
  P   P   G   W   H   V   V   L   G   G   L   W   L   Q   T   *   Q   A   V   H
61/21                                                  91/31
ATG CAC TGT CCA CGT AAG TTC CGC TTA CAA AAA ATT TGG TTG TAC AAG CAA TAC AGA GAG
M   H   C   P   R   K   F   R   L   Q   K   I   W   L   Y   K   Q   Y   R   E
 C   T   V   H   V   S   S   A   Y   K   K   F   G   C   T   S   N   T   E   S
  A   L   S   T   *   V   P   L   T   K   N   L   V   V   Q   A   I   Q   R   V
121/41                                                 151/51
TAA GAG TAC ACA TCT CGA TGA CTT ACC TGC TGT GAT TTA ATA TTT CAG ATA TAC CGA GAA
*   E   Y   T   S   R   *   L   T   C   C   D   L   I   F   Q   I   Y   R   E
 K   S   T   H   L   D   D   L   P   A   V   I   *   Y   F   R   Y   T   E   K
  R   V   H   I   S   M   T   Y   L   L   *   F   N   I   S   D   I   P   R   S
181/61                                                 211/71
GTT GAA ACT CCT GGA AGA AAC AGT TTA ATC TAC CAC TCA ATA ACC AAG AAG GGA CCT TTG
V   E   T   P   G   R   N   S   L   I   Y   H   S   I   T   K   K   G   P   L
 L   K   L   L   E   E   T   V   *   S   T   T   Q   *   P   R   R   D   L   C
  *   N   S   W   K   K   Q   F   N   L   P   L   N   N   Q   E   G   T   F   A
241/81                                                 271/91
CAT GAA ACC CCA ATC AGT GAT CAA TAT AAG CCC CTG GGA TAT CTC GAC AGG CAA CGT TTA
H   E   T   P   I   S   D   Q   Y   K   P   L   G   Y   L   D   R   Q   R   L
 M   K   P   Q   S   V   I   N   I   S   P   W   D   I   S   T   G   N   V   *
  *   N   P   N   Q   *   S   I   *   A   P   G   I   S   R   Q   A   T   F   S
301/101                                                331/111
GCA GCA AGG AGG AGT AAC ACT ACT TAT TGC TAT GAC TTC CCG TTG GTT TGT TAC T
A   A   R   R   S   N   T   T   Y   C   Y   D   F   P   L   V   C   Y
 Q   Q   G   G   V   T   L   L   I   A   M   T   S   R   W   F   V   T
  S   K   E   E   *   H   Y   L   L   L   *   L   P   V   G   L   L
```

~~The sense strand of the Arabidopsis sequence for pKLU81 with 3 Phase translation shown. The sequence shown is at the 3' end of the subclone. The 1st phase is the translation from the largest open reading frame.~~

FIGURE 10.

~~Arabidopsis 5' translated open reading frame FASTA against SWISSPROT Data base Scores shown are the two best fit~~

```
SCORES      Init1: 87 Initn: 139 Opt: 125
              38.4% identity in 73 aa overlap

                        10        20        30        40        50
Arab                RPYFLQAAVPLLYPCTRIMYCLFLFNFVEIRRFYGIEHGGGYDSWRKTSVVAF
                                                 ||:: :||::||:       ::| ::
Rat       PRLQVEHPCTEMVADVNLPAAQLQIAMGIPLFRIKDIRMMYGV------SPWGDA-----
        440       450       460       470       480

              60        70        80        90       100       110
Arab      PFDFDKAQSIR-PKGHCVAVRVTSEXPDDGFKPTSGRVQVMZYLWNAKZKFIHZGTLWGN
          |:||:::: :: |:|| :|:|:|||:||:||||:|| || :::
Rat       PIDFENSAHVPCPRGHVIAARITSENPDEGFKPSSGTVQELNFRSNKNVWGYFSVAAAGG
        490       500       510       520       530       540

              120       130       140       150       160       170
Arab      TCMNLQQXLSFKSKPNVWAYFSVKVIYIYXXSAIZVFHNXLIXRLLFYSCGAPXSWFHLE

Rat       LHEFADSQFGHCFSWGENREEAISNMVVALKELSIRGDFRTTVEYLIKLLETESFQLNRI
        550       560       570       580       590       600
```

```
SCORES      Init1: 87 Initn: 116 Opt: 117
              46.5% identity in 43 aa overlap

              30        40        50        60        70        80
Arab      LFNFVEIRRFYGIEHGGGYDSWRKTSVVAFPFDFDKAQSIR-PKGHCVAVRVTSEXPDDG
                                               ::||:::: :: |:|| :|:|:|||:||:|
Chick     AAQLQIAMGIPLHRIKDIRVMYGVSPWGDGSIDFENSAHVPCPRGHVIAARITSENPDEG
        460       470       480       490       500       510

              90        100       110       120       130       140
Arab      FKPTSGRVQVMZYLWNAKZKFIHZGTLWGNTCMNLQQXLSFKSKPNVWAYFSVKVIYIYX
           |||:|| || :::
Chick     FKPSSGTVQELNFRSNKNVWGYFSVAAAGGLHEFADSQFGHCFSWGENREEAISNMVVAL
        520       530       540       550       560       570
```

FIGURE 11A-D

A
Yeast ACCase Domain's
BIOTIN CARBOXYLASE DOMAIN
BIOTIN BINDING DOMAIN
TRANS CARBOXYLASE DOMAIN
Area :-
A
Aii
B
C
D
E
F
Arabadopsis Genomic ACCase gene (19 kb)
B
i
Area Aii ORF->
Yeast ACCase ->
ii
Area C ORF->
Arab
Yeas
iii
Area E DNA sequence
Area F DNA sequence
Rape ACCase cDNA
2000 bp
2000

Figure 11c Region A.

TCGACTCGATCTGAAAATATCTAGTGTTCAACAAACTTCAGATTCTTCGATCTACATATAAAT
CTGTTACATTCTTTTTTTTATCAAAGAAATCACATTATTTTAGTAACTAATCCTAACTATAAA
ATCTTTATTCAAGTATTTGATTATCCTTGATGAACTTTTAACAAACGGAATCAAATATAGGAA
ACTAAATCGACCTATACAGAAAAATAATATTTAAATACAATACTTTTTTTTCCTACTTAGCAC
TTGGATGGCTTTATTGGCTTCATGATCTAGTGGAGCAAGATCAGTAGAGATTTGATATGGTT
CAAGTTTGTTCTGGTCTAGTTTTTACGGGCATTTTTATGTACCTCGTGAACTTTCAAGTTATA
AAATCCCGGTGCCTTGGAAAAAAAAAGGTCTCAAAGACATAAGCATACAATAAAATTTGTTT
TACAAAGTTTGGAACAAGTCAACGATGATTCGTTAATTTTCATTGCTAAAATGATTGGATCA
TTCACAATTAACAAAAATGAGGAAAGAATGAGAGAAAGATGATAAGGTTGCCATACAATA
TAAACCCATACCTAACTCTCAACTATATCTCAACCCCCAGTCATTTATAGTTACTATTAAGCC
ATTAATATTATTTCTTTGTCAATGAGACCACTTTTATTCTCATTTTAAATAATCAAACAAAAT
GAAGAT

Figure 1 - Region #iii)

GAACTACTATTATCTGAATTAACCGTGTTTTACTGTACAGAACACATGTATTAAGCTCAATTT
CAGCAATGAAGTTTTGGTCTTTGGAGTTATTTGTCATTCATCTGAACATCTTTGTCTACAACC
TGTGTGCAGATGGCTGAAGTAACACGCGTGGATGCAGTTTGGCCTGGTTGGGGTCATGCAT
CTGAAAACCCCGAATTACCTGATGCCCTAGATGCAAAAGGAATCATATGTCTTGGTCCTCCA
GCATCTTCAATGGCAGCACTGGGAGATAAGATTGGTTCTTCGTTGATTGCACAAGCTGCTGA
TGTACCCACTCTGCCATGGAGTGGTTCCCATGTAAGTAAATTTACTCTTGTTAAGCTTGAGTA
TTCTATAGTGTCACCTAAATA

Figure 5

GGAGGGTCCAATTACTGTGCTCCGCCAGAAACTTTTCAAGAAACTTGAACAAGCAGCTAGA
AGGTTGGCTAAGAGTGTTAACTATGTTGGAGCTGCTACTGTTGAGTATCTCCACAGTATGGA
CACTGGGGAGTACTACTTCTTAGAGCTTAACCCTCGCTTACAGggtggtttcatactgcagc
ttttgcgttgaaatataatgaaggtccggacttgaaaattgaatgacttgtttaacttgat
gtttgagGTCAGGTTGAGCATCCTGTCACTGAGTGGATTGCCGAGATAAATCTTCCTTCTGCCCA
AGATATACTGTGGGGATGGGAATTCCTCTCTGGCAAATCCCTGGTATAATCTACGTCCTTAT
TTCTTACAGgcagcggttcctcttctttatccatgcacacgaataatgtactgtctgtttct
ctttaatttcgtagagataagacggttctatggaatagaacatggtggagGTTATGATTCTT
GGCGAAAAACATCTGTTGTAGCCTTCCCTTTTGATTTTGATAAAGCTCAATCTATAAGGCCA
AAAGGTCATTGTGTGGCTGTACGTGTGACAAGTGAGGATCCTGATGACGGGTTCAAACCAA
CCAGCGGTAGAGTTCAGgtaatgtgatatctgtggaatgcaaagtgaaagttcattcactg
agaactctgtgggtaacacttgtatgaacttgcaacagGAGTTGAGTTTTAAGAGCAAGCC
AAATGTGTGGGCGTACTTCTCTGTCAAGgtaattatatctatagagactctgctatataag
tgtttcacaatgttttaaattttacgactacttttttacagtctggtggagGCATCCACGA
GTTCTCG

Figure 1 - Figure 2

ctatgtaagaacctctttctcagagATTTATTTGTCTTGAAAAGTTTCTATCTGGTGACGA
AATGTTCTATCTGTCCAGAAAGCATCAGCGACCAGTGCTGCTGTGGTTTCAGATTACGTTGG
TTATCTGGAGAAGGGGCAAATCCCTCCAAAGgtaatccaataccagggatctctttgcctt
tctagtgatgttcttgtagctaacttttctctcttaacttgcagcatatatctcttgtaca
ttctcaagtgtctctgaatattgaagGAAGTAAATATACGGTATTCGCCTACTATCCAAATT
TTACGTCTCTGCAATTTCGTATTTTCCTCTGCCATATTATTTTTGCGCTGAAGATATTGTTA
CCAGgcttactaacatgaacataactgttctagagtgattagcaatgtagtccgggggtggat
cagGAACCTACAGGCTAAGAATGAACAAGTCAGAAGTGGTAGCAGAAATACACACTCTACGT
GATGGAGGTCTGTTGATGCAGgcaagtttctgccttttctatactacaagacaaggacat
acatgtgtcgcgcagaaaaaaacttctggagaatctcacttccttttcttgtttcactgtc
attgcagttggatggcaaaagccatgtgatatatgcagagGAAGAAGCTGCAGGAACTCGTC
TTCTCATTGATGGAAGAACTTGTTTGCTACAGgtttctgctaatttttttgtgtgtttacca
tttacttcacgtttctctgaagtcatctttagcttttaagctgtctgtcaattttggctta
ttcagAATGACCATGATCCATCAAAGTTAATGGCTGAGACACCGTGCAAGTTGATGAGGTAT
TTGGTTTCTGACAACAGCAATATTGACGCTGATACGCCTTATGCCGAAGTTGAGGTCATGAA
GATGTGCATGCCACTTCTTTCACCTGCTTCAGGAGTTATCCATCTTAAAATGTCTGAAGGAC
AAGACATGCAGgttcacttcattgctaaacaaaaagtctacagttctgtttaaattgattaa
cccatccattatttttttcacagGCTGGTGAACTTATCGCCAATCTTGATCTTGATGATCCT
TCTGCTGTAAGAAAGGCCGAACCCTTCCATGGAAGTTTCCCAAGATTAGGGCTTCCAACTGC
AATATCCGGTAGAGTTCATCAGAGATGTGCCGCAACATTAAATGCTGCACGCATGATTCTTG
CTGGCTATGAGCATAAAGTAGATGAGgtaaacactgttgttttcctattgatccaactc
tctctactagattatttgactatgagatagctcatacgtcgcagGTTGTTCAAAGACTTACT
TAATTGCCTTGATAGCCCTGAACTCCCATTTCTTGCAGTGGCAACAGTGCTTTGCAGTTCTG
GCGACACGACTACCTAAAAATCTCAGgaacatggtaaacacctgtgtagtattcataatccg
gttcttatatattgatatttgttttgagttcaagacttttaatcatatctaaataaaactct
ttatcagctagaatcaaagtatagGGAATTTGAGAGTATTTCCAGAAACTCTTTGACCACCG
ATTTCCCTGCCAAACTTTTAAAAGGCAGTC

Figure 11c Kujan D.

CGAGTCAATTACTTGAACAGACCAAACTAAGTGAAGCTTCGTTCAAACATTGCTAGAAGCC
TTTCAGAGTTAGAAATGTTTACAGAGGACGGAGAAAATATGGATACTCCCAAGAGGAAAA
GTGCCATTAATGAAAGAATAGAAGATCTTGTAAGCGCATCTTTAGCTGTTGAAGACGCTCT
CGTGGGACTATTTGACCATAGCGATCACACACTTCAAAGACGGGTTGTTGAGACTTATATT
CGCAGATTATACCAGgttcgagttcattcttccgcacccttattgttcaaaattcttttttg
tactgcaattgattacagAAAATTTTGACTTCATTTTAACCCGACTCTTGTCATCAGCCCT
ACGTCGTTAAAGATAGCGTGAGgatgcagtcgcgccggatgcagtggcacctttctggtct
tcttgattcctgggatttcctagagGAGCATATGGAAAGAAAAAACATTGGTTTAGACGAT
CACGACACATCTGAAAAAGGATTGGTTGAGAAGCGTAGTAAGAGAAAATGGGGGGCTATG
GTTATAATCAAATCTTTGGAGTTTCTTCCACGTATAATACGTGCAGCATTGAGAGAAACAT
AGCACAACGACTATGAAACTGCCGGAGCTCCTTTATCTGGCAATATGATGCACATTGCTAT
TGTCGGGCATCAACAACCAGATGAGTCTGCTTCAGGACAGGTACTTGACACAGTAT

Figure . c Rujin E.

ACCGAGAAGTGAACCTGAAGAAACAGTTTAATCTACCACTCAATAACCCAAGAAGGGACCT
TTGCATGAAACCCCAATCAGTGATCAATATAAGCCCCTGGGATATCTCGACAGGCAACGTT
TAGCAGCAAGGAGGAGTAACACTACTTATTGCTATGACTTCCCGTTGgtttgttactgaat
tcataagattcacacatacgcttactctttggctatttccaaccccccttatgttatttc
tttcctttcagGCATTTGGGACAGCCTTGGAACTGTTGTGGGCATCACAACACCCAGGAG
TTAAGAAACCATATAAGGATACTCTGATCAATGTTAAAGAGCTTGTATTCTCAAAACCAGA
AGGTTCTTCGGGTACATCTCTAGATCTGGTTGAAAGACCACCCGGTCTCAACGACTTTGGA
ATGGTTGCCTGGTGCCTAGATATGTCGACCCCAGAGTTTCCTATGGGGCGGAAACTTCTCG
TGATTGCGAATGATGTCACCTTCAAAGCTGGTTCTTTTGGTCCTAGAGAGGACGCGTTTTT
CCTTGCTGTTACTGAACTCGCTTGTGCCAAGAAGCTTCCCTTGATTTACTTGGCAGCAAAT
TCTGGTGCCCGACTTGGGGTTGCTGAAGAAGTCAAAGCCTGCTTCAAAGTTGGATGGTCGG
ATGAAATTTCCCCTGAGAATGGTTTTTCAGTATATATACCTAAGCCCTGAAGACCACGAAA
GGATTGGTCATCTGTCATTTGCCCATGAAGGTAAAGCTCCCTAGTGGGGGAAACTAGGGTG
GGGTGAATTGATACGGTCGTTGGGCAAAGAAGGATGG

Figure 1

gcaagctcgaaattaaccctcactaaagGGAACAAAAGCTGGAGCTCTCTTGTAAAAAACG
TAAGAGAAGCATCTGGAGACAACTTAGCATATAAATCTTCAATGCGTCTGATTCAGGATTG
GTTCTGCAACTCTGATATTGCAAAGGGGAAAGAAGAAGCTTGGACAGACGACCAAGTGTTC
TTTACATGGAAGGACAATGTTAGTAACTACGAGTTGAAGCTGAGCGAGTTGAGAGCGCAGA
AACTACTGAACCAACTTGCAGAGATTGGGAATTCCTCAGATTTGCAAGCTCTGCCACAAGG
ACTTGCTAATCTTCTAAACAGggtataaaacaaaaccccccaaaaaaacaaggttttggtc
cccaagtaatcctaacctgtatgccggttttaaagccctaagtaaatatttgtgatgcaG
GTGGACCGTCGAAAAAGAGAAGAGCTGGTGGCTGCTATTCGAAAGGTCTTGGGTTGACTGA
TATCGAAGACTTTAGCTTCTAATCCAAGAAAGATGGACATTTAAAGTTTGCTTGTGTCCAT
TTGGACCATCTTCCTTATATTTGTTGGTCACAGTTGTAAATGTTGTTGTAGCTTTGTCATT
TCCGTATAAACAAATTACGCAATAATTCATTCAACATGTCACTCTTGCTTCATATTTATAC
ACTGAACCAAGACAATATAATAGTCTAAATATAAAACTGATCGGTCGACGCCCTATAGTGA
GTCGTATTAAGCCGGCCGCGAGCTCTAGAGTC

FIGURE 12.

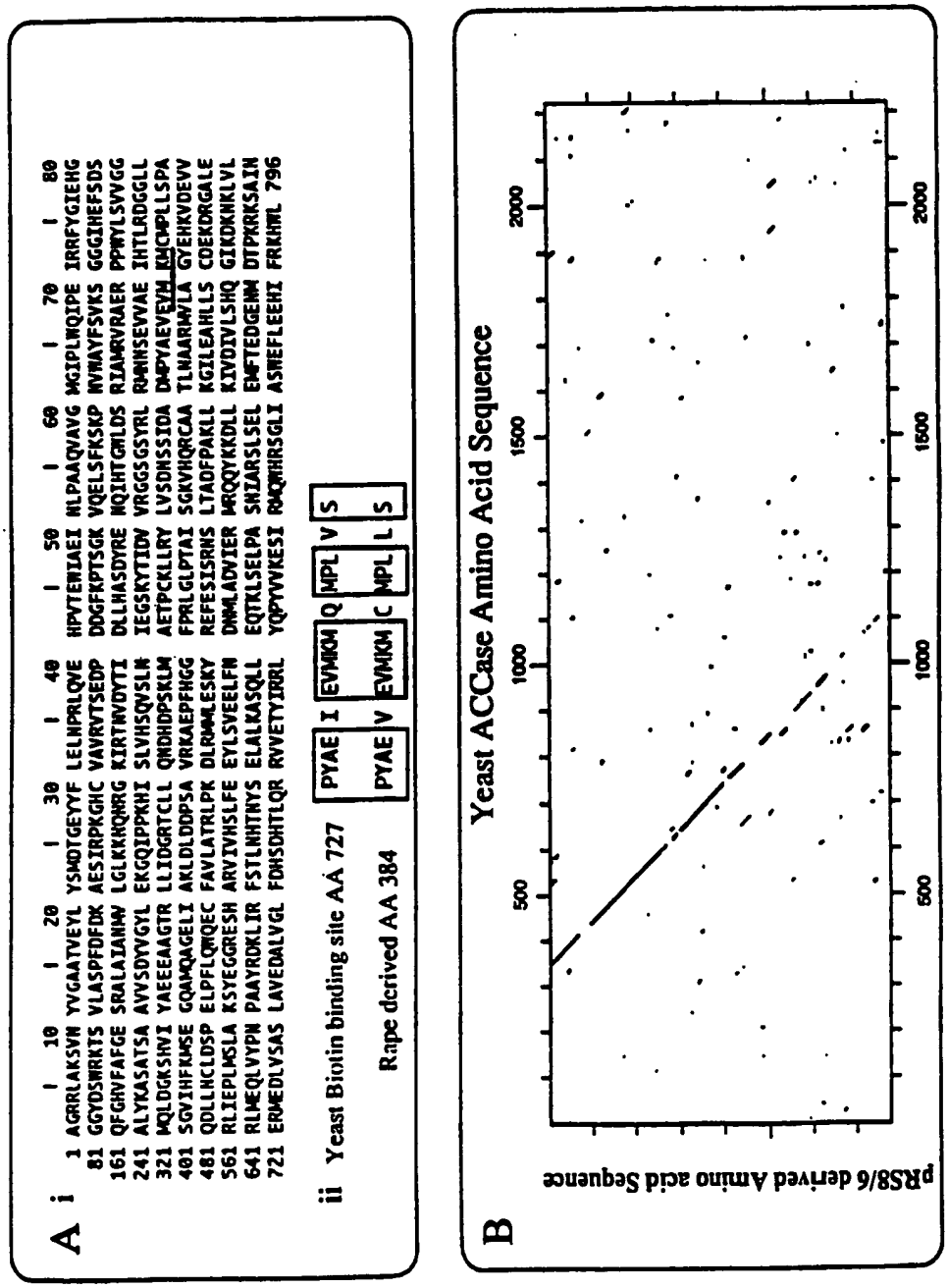

A
i
1 AGRRLAKSVN YVGAATVEYL YSWDTGEYYF LELNPRLQVE HPVTEMIAEI HLPAAQVAVG MGIPLWQIPE IRRFYGIEHG
81 GGYDSWRKTS VLASPFDFDK AESIRPKGHC VAVRVTSEDP DDGFKPTSGK VQELSFKSKP NVMAYFSVKS GGGIHEFSDS
161 QFGHVFAFGE SRALAIANMV LGLKKHQNRG KIRTNVDYTI DLLHASDYRE NQIHTGWLDS RIAMRVRAER PPMYLSVVGG
241 ALYKASATSA AVVSDVVGYL EKGQIPPKHI SLVHSQVSLH VRGGSGSYRL RWNNSEVVAE IHTLRDGGLL
321 MQLDGKSHVI YAEEEAAGTR LLIDGRTCLL QNDHDPSKLM AETPCKLLRY LVSDNSSIDA DAPYAEVFVH LMCWPLLSPA
401 SGVIHFKMSE GQAMQAGELI AKLDLDDPSA VRKAEPFHGG FPRLGLPTAI SGKVHQRCAA TLNAARMVLA GYEHKVDEVV
481 QDLLNCLDSP ELPFLQWQEC FAVLATRLPK DLRMMLESKY REFESISRNS LTADFPAKLL KGILEAHLLS CDEKDRGALE
561 RLIEPLMSLA KSYEGGRESH ARVIVHSLFE EYLSVEELFN DNMLADVIER MRQQYKKDLL KIVDIVLSHQ GIKDKHKLVL
641 RLMEQLVYPN PAAYRDKLIR FSTLNHTNYS ELALKASQLL EQTKLSELPA SMIARSLSEL EMFTEDGEMW DTPKRKSAIN
721 ERMEDLVSAS LAVEDALVGL FDHSDHTLQR RVVETYIRRL YQPYVVKESI RAKQWHRSGLI ASWEFLEEHI FRKHML 796
ii Yeast Biotin binding site AA 727 PYAE I EVMKM Q MPL V S
Rape derived AA 384 PYAE V EVMKM C MPL L S
B
Yeast ACCase Amino Acid Sequence
pRS8/6 derived Amino acid Sequence
500
1000
1500
2000

Figure 1.C

TGGCTGGTAGAAGGTTGGCTAAGAGTGTTAACTATGTTGGAGCAGCTACTGTTGAATATCTC
TACAGCATGGACACGGGGGAGTACTACTTCTTAGAGCTTAACCCTCGGTTACAGGTTGAGC
ACCCTGTAACTGAATGGATTGCCGAGATAAATCTTCCTGCTGCGCAAGTTGCTGTTGGGATG
GGAATTCCTCTCTGGCAAATCCCTGAGATAAGACGGTTCTATGGTATAGAACATGGTGGAGG
TTACGATTCTTGGAGGAAAACATCTGTGCTAGCCTCCCCTTTTGATTTTGATAAAGCTGAATC
TATAAGGCCAAAAGGTCATTGTGTGGCTGTACGCGTGACAAGTGAGGACCCTGATGACGGA
TTCAAACCCACCAGCGGTAAAGTACAGGAGTTGAGTTTTAAAAGCAAGCCAAATGTGTGGG
CTTACTTCTCTGTCAAGTCTGGTGGAGGCATCCACGAGTTCTCAGATTCCCAATTTGGCCATG
TTTTTGCATTTGGGGAATCCAGAGCCTTGGCAATAGCAAATATGGTCCTTGGGCTTAAAAAA
AATCAAAATCGTGGAAAAATTAGGACTAACGTTGACTACACGATTGACCTTTTACATGCTTC
TGATTACCGGGAAAACCAAATTCACACTGGTTGGTTGGACAGTAGAATTGCTATGCGGGTC
AGGGCAGAGAGGCCTCCATGGTACCTCTCTGTTGTCGGAGGGGCTCTCTATAAAGCATCAG
CGACCAGTGCTGCTGTAGTCTCGGATTATGTTGGTTATCTAGAGAAGGGACAAATTCCCCC
AAAGCATATATCTCTTGTGCATTCTCAAGTGTCTCTGAACATTGAAGGAAGTAAATATACGA
TTGATGTGGTCCGGGGTGGATCAGGAAGCTACAGGCTAAGAATGAACAACTCAGAAGTTGT
AGCAGAAATACACACTCTACGTGATGGAGGTCTGTTGATGCAGTTGGATGGTAAAAGCCAT
GTGATATATGCAGAGGAAGAAGCTGCAGGAACCCGTCTTCTTATTGACGGAAGAACTTGTT
TACTTCAGAATGATCACGATCCTTCAAAGTTGATGGCTGAGACACCGTGCAAGCTGCTGAG
GTATTTGGTTTCAGATAATAGCAGTATTGATGCTGACATGCCCTACGCGGAAGTTGAGGTCA
TGAAGATGTGCATGCCACTTCTTTCACCTGCATCAGGAGTTATACATTTCAAAATGTCTGAA
GGACAAGCCATGCAGGCTGGTGAACTTATAGCCAAGCTTGATCTTGATGATCCTTCTGCTGT
AAGAAAGGCCGAACCCTTCCATGGAGGTTTCCCAAGATTAGGGCTTCCAACGGCAATTTCTG
GTAAAGTTCATCAGAGATGTGCTGCAACTTTAAATGCTGCTCGCATGGTTCTTGCCGGCTAT
GAGCATAAAGTAGATGAGGTTGTTCAAGACTTGCTTAACTGCCTTGATAGCCCTGAACTCCC
ATTCCTTCAGTGGCAAGAGTGCTTCGCAGTTCTGGCAACACGACTACCGAAAGATCTCAGAA
TGATGTTAGAATCCAAGTATAGGGAATTTGAGAGTATATCCAGGAACTCTCTCACCGCAGAT
TTCCCTGCCAAACTTTTAAAAGGCATTCTTGAGGCTCATTTATTATCTTGTGATGAGAAAGAT
AGGGGTGCCCTTGAAAGGCTCATTGAACCATTGATGAGCCTTGCAAAGTCTTATGAAGGTG
GTAGAGAAAGTCATGCCCGTGTTATTGTTCATTCTCTTTTTGAAGAATACCTATCTGTAGAA
GAATTATTCAATGATAACATGCTGGCTGATGTTATTGAACGCATGCGTCAGCAATACAAGAA
AGATCTGTTGAAGATTGTTGATATTGTGCTCTCACACCAGGGCATTAAAGACAAAAACAAAC
TCGTTCTTCGGCTCATGGAGCAGCTTGTTTACCCTAATCCTGCTGCATACAGAGATAAACTTA
TCCGATTCTCGACACTAAACCATACTAATTACTCTGAGTTGGCACTGAAGGCAAGCCAATTA
CTCGAACAGACCAAATTAAGTGAACTTCCAGCTTCAAACATTGCTAGAAGCCTGTCAGAGTT
AGAAATGTTTACAGAGGATGGGGAAAATATGGATACTCCCAAGAGGAAGAGTGCCATTAA
TGAAAGAATGGAAGATCTTGTGAGCGCATCCTTAGCTGTTGAAGATGCTCTCGTGGGACTA
TTTGACCACAGCGATCACACACTTCAAAGACGAGTTGTTGAGACTTATATTCGCAGATTATA
TCAGCCCTACGTCGTCAAAGAAAGCATCAGGATGCAATGGCACCGGTCTGGTCTTATTGCTT
CTTGGGAGTTCCTAGAGGAGCATATTTTCCGGAAACATTGGCTTA

FIGURE 13.

~~ACCase Southern Blot Analysis of Rape and Arabidopsis genomic DNA~~

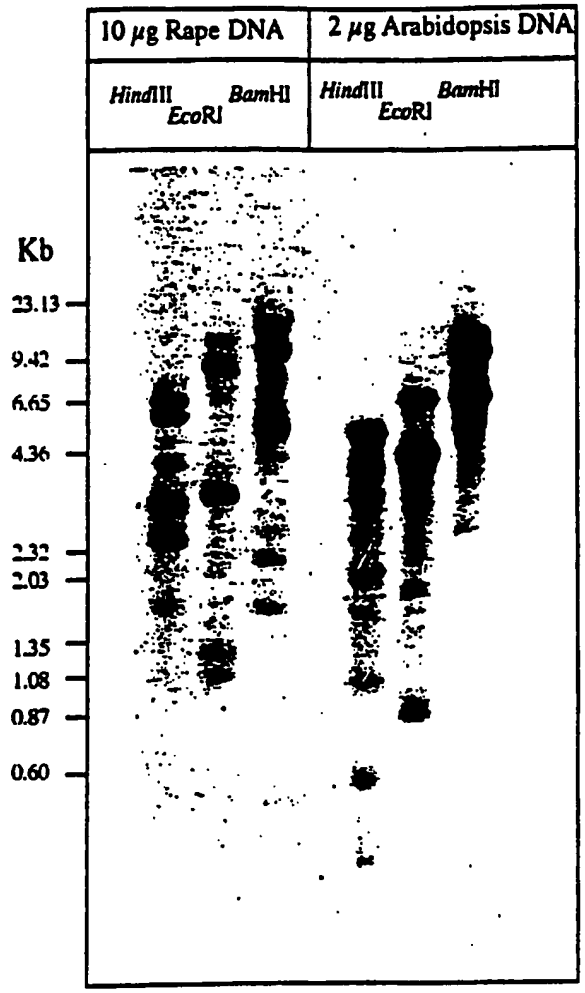


FIGURE 14.

~~Fatty Acid Profile and Northern Blot Analysis of Rape Embryogenesis~~

Fig 14A

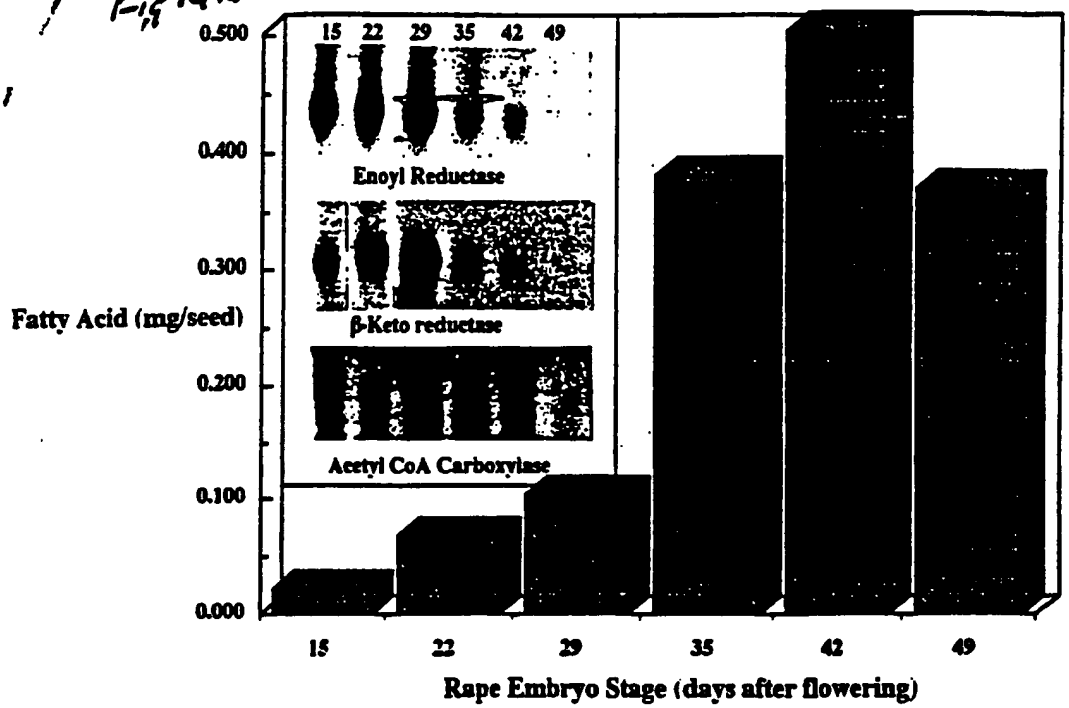
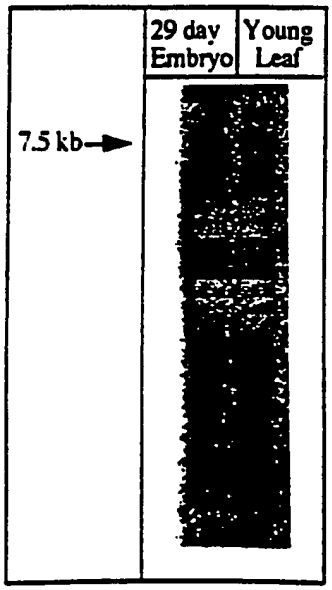


Fig 14B

~~B Northern Blot analysis of ACCase and Rape embryo-V leaf expression~~



INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/GB 94/00846

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/52 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 119, no. 21, 1993, Columbus, Ohio, US; abstract no. 221570, SLABAS, A.R., ET AL. 'Biochemistry and molecular biology of lipid biosynthesis in plants: potential for genetic manipulation' see abstract & PLANT BIOTECHNOLOGY 3(BIOSYNTHESIS AND MANIPULATION OF PLANT PRODUCTS), 1993 pages 104 - 138 --- -/--	1,4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
21 December 1994	01.02.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Maddox, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLANT MOLECULAR BIOLOGY, vol.24, no.1, January 1994 pages 21 - 34 ELBOROUGH, K.M., ET AL. 'Studies on wheat acetyl CoA carboxylase and the cloning of a partial cDNA' see the whole document	2,4
O,X	J. CELL. BIOCHEM. SUPPL., KEYSTONE SYMPOSIUM ON IMPROVED CROP AND PLANT PRODUCTS THROUGH BIOTECHNOLOGY, JAN. 9-16, 1994., vol.18A, 1994 page 113 ELBOROUGH, K.M., ET AL. 'Regulation of primary storage products of oil seeds by manipulating the levels of the genes involved in lipid metabolism on plant acetyl CoA carboxylase' see abstract X1-418	1,2,5, 8-11
X	WO,A,93 11243 (ICI) 10 June 1993 see the whole document	5-11,14, 15
X	WO,A,94 08016 (ARCH DEVELOPMENT CORP.) 14 April 1994 see claims 28-40	5,8,9, 13-15
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